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A QUALITATIVE STUDY OF THE BACTERIAL FLORA OF SEA AND LAND BREEZES

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The studies of Burke (1934) indicate that fresh or brackish water may serve as an intermediary for the interchange of bacteria between the land and the sea. This paper is the first of a series which is concerned with the air as the vehicle for the interchange of marine and terrestrial bacteria and incidentally the observations may prove to have some meteorological significance as discussed below.

While the atmosphere is probably not an important disseminator of human pathogens, certain microörganisms may be conveyed great distances by air. Proctor (1935) found numerous living bacteria at all altitudes at which the air was examined, up to 20,000 feet, where they have almost limitless possibilities of travel in a horizontal direction. Snow (1926) recovered many viable bacteria from wind-blown desert soil of Arizona, as did Brown (1930) from the atmosphere, thereby demonstrating tolerance of air-borne bacteria for radiations, heat and desiccation.

Experimental.—Since it has been shown by Lipman (1926), Korinek (1926) and others that marine bacteria differ from fresh-water varieties in their ability to grow on sea-water or fresh-water media, these two kinds of nutrient media were used for the detection of air-borne bacteria. According to ZoBell and Feltham (1933) less than five per cent of the bacteria in sea water will multiply in fresh-water nutrient media and a smaller percentage of fresh-water bacteria will grow on sea-water media. Soil bacteria are more euryhaline. The nutrient-media consisted of 0.3 per cent Bacto-peptone, 0.2 per cent proteose-peptone, 0.3 per cent beef-extract, 0.025 per cent $FeCl_3.6H_2O$ and 1.5 per cent agar. In half of the media distilled water was used as the solvent and in the other half sea water was used. These will be referred to as fresh-water (*FW*) and sea-water (*SW*) media, respectively. The media were adjusted to pH 7.5 and sterilized at 120°C. for 20 minutes. Shortly before use 10 to 15 ml. of the medium was permitted to solidify in sterile Petri dishes. By removing

the covers of the dishes the media were exposed to the air for periods of time ranging from fifteen minutes to two hours in an effort to obtain from 30 to 300 colonies per plate. Plates of the two kinds of media were exposed in duplicate under comparable conditions to the air during gentle sea and land breezes which in most cases had been blowing in that direction for several hours prior to the exposure of the plates. Some of the plates were exposed at the sea side, some inland and others at sea on the boat *Scripps*. The exposed plates were incubated at 25°C. and the colonies were enumerated after four to seven days or when the counts approached constancy.

In the first series of observations the two kinds of media (*FW* and *SW*) were exposed atop the super-structure of Ritter Hall, a three-story Scripps

TABLE 1

NUMBER OF BACTERIA WHICH DEVELOPED ON CORRESPONDING SEA-WATER (*SW*) AND FRESH-WATER (*FW*) NUTRIENT AGAR PLATES WHICH WERE EXPOSED TO SEA AND LAND BREEZES ATOP RITTER HALL AT LA JOLLA. THE RATIO OF THE *SW*:*FW* COUNTS ARE ALSO GIVEN

DATE OF EXPOSURE	DIRECTION OF WIND	VELOCITY OF WIND	BACTERIA PER PLATE *SW* MEDIUM	BACTERIA PER PLATE *FW* MEDIUM	RATIO *SW*/*FW*
5/16/35	off-sea	5.4 m. p. h.	138	34	4.06
6/5/35	off-sea	7.4 m. p. h.	206	39	5.28
6/6/35	off-sea	3.0 m. p. h.	154	43	3.58
9/1/35	off-sea	8.3 m. p. h.	285	22	12.96
Average of 19 sets of plates	off-sea	5.6 m. p. h.			*8.61*
6/17/35	off-land	1.1 m. p. h.	112	240	0.46
6/21/35	off-land	0.9 m. p. h.	52	97	0.35
9/25/35	off-land	2.6 m. p. h.	64	294	0.22
9/26/35	off-land	4.5 m. p. h.	179	262	0.68
Average of 14 sets of plates	off-land	3.8 m. p. h.			*0.43*

Institution building located about 200 meters from shore. Aseptic precautions were exercised to avoid contaminating the media with any bacteria except those which might fall in from the air. Table 1 gives representative data showing the number of bacteria which developed on the two kinds of media after identical exposures to the air during either sea or land breezes. On the nineteen sets of plates which were exposed to sea breezes there were from 2.7 to 55 times as many bacteria which developed on the *SW* medium as on the corresponding *FW* medium, there being an average of 8.7 times more on the *SW* medium. Conversely on the fourteen sets of plates which were exposed during land breezes the *SW* counts were only from 0.17 to 0.78 times as high as the *FW* counts, the average being 0.43.

Besides differing in their salinity requirements, nearly all of the marine

bacteria are Gram-negative, whereas about half of the terrestrial bacteria (*FW* medium) are Gram-positive. The average cell size of the former is smaller than the latter. Yeasts and actinomyces which are included in the bacterial counts occur frequently on the *FW* medium exposed to land breezes and only rarely in sea breezes or on *SW* medium. Molds appeared on both kinds of media but fewer on the *SW* than on the *FW* medium and fewer in sea than in land breezes. Cocci, as molds, yeasts and actinomyces, are rarely found in the sea nor were they recovered from sea breezes as often as in land breezes. A somewhat larger proportion of the air-borne terrestrial bacteria are sporogenic than of the marine forms, the majority of the latter being asporogenic. As a rule the marine bacteria form smaller colonies which develop more slowly than the terrestrial bacteria. Likewise a larger percentage of the marine bacteria are agar liquefiers.

The ratio of the *SW*:*FW* counts on plates exposed during land breezes are within the range of the *SW*:*FW* counts on soil samples which indicates, as may be expected, that most of the bacteria carried by land breezes are of terrestrial origin. While the *SW*:*FW* counts on plates exposed during sea breezes are considerably lower than the *SW*:*FW* counts of sea-water samples, they do indicate that a large proportion of the bacteria in sea breezes are probably of marine origin. Many but not all of the latter find their way into the air via the surf spray along the beach which in front of the Institution is sandy and shallow, permitting the large rollers from the open ocean to form huge breakers. When one considers that the entire ocean surface is constantly agitated and stirred by wind and breakers, it is only logical to assume that some sea water containing bacteria is suspended in the atmosphere from the open ocean. Proctor (1935) has shown that through the chance action of air currents, water and soil bacteria may attain altitudes of 20,000 feet or more.

The effect of the surf spray on the bacterial content of the air near the sea side was evaluated by exposing sets of plates under different conditions of tide and wind direction along the railing of the pier which is from 6 to 8 meters above the water level and extends 325 meters from shore. In general, the largest total *SW* counts as well as the highest *SW*:*FW* ratios were obtained on the leeward side of the breakers regardless of whether the wind was off-land or off-sea. Even within the length of the pier the *SW* counts decreased rapidly with distance from the breakers, the distance depending primarily upon the wind velocity. However, the *SW* counts exceeded the *FW* counts on most sets of plates exposed during gentle land breezes atop the observation house on the end of the pier two or three hundred meters seaward from the surf. This indicates that either many marine bacteria are being blown into the air with the spray or else the water droplets from the spray tend to precipitate bacteria from the air which are already suspended. Both probably occur as illustrated by the data in

table 2 which show that the largest *FW* as well as *SW* counts occur near the surf zone.

The influence of the littoral surf spray on the bacterial content of the air does not extend 5 miles to sea during land breezes as shown by the *SW*:*FW* ratios of less than unity. Several sets of plates were exposed at sea on the forecastle of the boat *Scripps* at distances ranging from 5 to 130 miles from the mainland. On two cruises when land breezes prevailed the *FW* counts exceeded the *SW* counts at all stations, the farthest of which was nearly a hundred miles from the mainland. On these two cruises the

TABLE 2

NUMBER OF BACTERIA PER SQUARE FOOT PER HOUR WHICH DEVELOPED ON COMPARABLE SEA-WATER (*SW*) AND FRESH-WATER (*FW*) MEDIA EXPOSED TO AIR DURING A SEA BREEZE HAVING A VELOCITY OF 5.8 MILES PER HOUR

	DISTANCE FROM SEA-WALL IN METERS					
	0	100	200	400	800	1600
Bacteria on *SW* medium	4610	5480	2630	1740	1280	490
Bacteria on *FW* medium	234	472	1090	1570	1830	1060
Ratio of *SW*:*FW* count	19.7	11.6	2.63	1.09	0.71	0.46

average *SW*:*FW* count ratio was 0.57. On three cruises when the prevailing direction of the wind was landward the *SW* counts exceed the *FW* counts. The average *SW*:*FW* count ratio was 2.37. A compilation of all the data collected at sea shows that the *SW* counts are on an average 1.64 times higher than the *FW* counts. This indicates the occurrence of many marine bacteria in the atmosphere over the sea. Most of the observations at sea were made during periods of relatively calm weather when the wind velocity was usually less than three miles per hour and the direction was frequently changeable although landward breezes predominated. Incidentally it was necessary to expose both kinds of media from four to eight times as long at sea as on land to give the same number of bacteria per plate during similar meteorological conditions. It should be borne in mind that the experimental procedure measures only the number of viable bacteria which are being precipitated from the air and detects neither those dead nor those which are stably suspended.

Many complications are encountered when an effort is made to determine how far inland marine bacteria are carried by the wind. Updrafts, turbulence, convections, advections and the precipitation of marine bacteria will tend to dilute the latter in the air as the distance from the sea increases. Also terrestrial organisms will be suspended in the air of sea breezes as they blow inland over the soil and through the vegetation. This is illustrated by the data in table 2 which gives the *SW* and *FW* counts at increasing distance from the sea when plates were exposed near the ground. Marine bacteria predominated only the first few hundred meters

from the sea side as indicated by the salt tolerance of the bacteria in the air.

On an inland trip during a sea breeze ranging in velocity from 5 to 12 miles per hour, plates were exposed on top of a car at nine different stations along the paved highway 6 to 80 miles from the coast resulting in an average *SW*:*FW* ratio of 0.524.

When plates are exposed at greater elevations it can be demonstrated that marine bacteria predominate in the air of sea breezes for some distance inland. Several sets of plates were exposed on the observation tower on Mt. Soledad which is a mile from the sea and about 850 feet above sea level. As on Ritter Hall, it was found that during sea breezes the *SW*:*FW* ratios exceeded unity whereas during land breezes the ratios were invariably less than unity. On one set of plates exposed during a sea breeze the day after a rainstorm, on the observation tower on Mt. Woodson which is 20 miles inland and has an elevation of 2890 feet, the *SW*:*FW* ratio was 2.06 thereby indicating a preponderance of marine bacteria in the air.

Whether or not marine bacteria will continue to predominate in the atmosphere at considerable distance inland above the zone of terrestrial agitation can be determined only by aerial ascensions to make observations under known meteorological conditions. Landsberg (1934) reports that while many condensation-nuclei are sodium chloride crystals, the number of such nuclei from the ocean is surprisingly small. However, a survey of the literature by Jacobs and Fleming (1936), as well as their own analytical data, shows that the chloride:sulfate ratio is higher in air masses of marine origin that in those of continental origin. The foregoing observations suggest that the bacterial content of the air likewise may serve as a criterion to aid the meteorologist in tracing the course and origin of large air masses.

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Summary.—The wind is an important factor in the interchange of bacteria from land and sea. In these preliminary investigations marine bacteria have been detected in the atmosphere 30 miles inland and terrestrial forms 130 miles at sea. The fact that about half of the soil bacteria are euryhaline while less than five per cent of sea-water bacteria are endowed with this property indicates that few terrestrial bacteria survive in the sea although many marine forms may survive in the soil. The studies suggest that the bacterial content of the atmosphere may aid the meteorologist in determining whether air masses are of marine or continental origin.

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GENETIC EFFECTS OF ULTRA-VIOLET RADIATION IN MAIZE. I. UNFILTERED RADIATION

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The genetic effects of ultra-violet light are of interest chiefly because of the possibility of differences in the effects of different wave-lengths. Comparison of x-rays of different wave-length range, reported by various investigators, has shown no qualitative differences in genetic effect. If the diverse genetic effects induced by radiation are dependent on different initial alterations in the irradiated material, it is more probable that distinctive wave-length relations of specific genetic alterations might be found in the ultra-violet than in the x-ray spectrum. Since more is known of the chemical effects of ultra-violet radiation than of x-ray, and since monochromatic radiations may be compared, it is possible that the investigation of variations in genetic effect between wave-lengths of the ultra-violet spectrum may ultimately provide a sounder basis for speculation as to the physical nature of induced mutation.

It is known from the studies of Altenburg,[1] Noethling and Stubbe[2] and others, that ultra-violet radiation induces mutation. The bactericidal effects of monochromatic ultra-violet radiations, as determined by numerous investigators (see Duggar[3]), show characteristic variations in the effectiveness of different wave-lengths, which, according to Gates,[4] parallel the absorption curves for certain derivatives of the nucleo-proteins. These substances, as well as various other proteins which have been studied,

show little absorption above 3100 Å. If genetic alterations induced by ultra-violet radiation are dependent upon absorption by proteins, therefore, it is probable that a limiting wave-length for genetic effectiveness may be found in the ultra-violet.

The chief obstacle to a survey of genetic effectiveness in this region of the spectrum is the difficulty of reaching the germ plasm with ultra-violet light. Altenburg's experiments with irradiation of mature flies showed that little, if any, of the radiation reaches the germ cells. The irradiation of the polar cap cells of the Drosophila egg, as developed by Geigy[5] and by Altenburg, makes possible the effective treatment of chromosomes of the germ line, but the rather involved genetic technique required probably precludes the use of this method in extensive comparisons of effects of specific wave-lengths. Penetration difficulties are less serious in the treatment of pollen, but there are few plant species in which genetic conditions are sufficiently favorable to make possible the extensive production and efficient classification of genetic alterations on the scale required.

Maize has unusual advantages for this sort of investigation, particularly in the numerous endosperm characters which may be used for the immediate indentification of deficiencies in the seeds produced by the use of treated pollen. Comparisons of the effects of radiations of different wave-length on the occurrence of endosperm deficiencies may be made quickly and almost unlimited numbers of individuals may be examined for the determination of critical regions of the spectrum. Detailed analysis of effects of radiation on the frequency of other genetic alterations may then be confined to the critical regions.

The genetic technique for the identification of germinal alterations in maize other than deficiencies is also fairly simple. The technique used is similar to that followed in experiments with x-ray treatment of pollen.[6] Mature pollen of a multiple dominant stock is treated and applied to silks of a multiple recessive. The occurrence of induced deficiencies at the marked loci is indicated in the F_1 seeds and plants by the appearance of the recessive characters. The occurrence of additional deficiencies and of translocations is shown by the segregation of defective pollen in the F_1 plants. Deficiencies and translocations are distinguished and genetically located by simple additional tests. Induced mutations are identified in the F_2 seeds and seedlings from the self-fertilized F_1 plants.

The experiments here reported were designed for the preliminary determination of the types of genetic effect produced in maize by ultra-violet treatment of pollen, and the identification of regions of varying effectiveness in the ultra-violet spectrum. It is planned to continue the study with monochromatic radiations. The results of the preliminary experiments indicating the genetic effects of different wave-lengths are summarized in this and the two accompanying papers. Genetic evidence on the induced

alterations will be reported in detail in a research bulletin of the Missouri Agricultural Experiment Station.

Results with Unfiltered Radiation.—The radiation here referred to as "unfiltered" ultra-violet is the radiation from a commercial quartz mercury-vapor arc (Cooper-Hewitt type *EAC*) operated at 4.8 amperes and 110 volts a. c. The treating distance was 19 cm. The pollen was irradiated in a single layer in a Petri dish, which was covered by a sheet of thin Cellophane. The filtering effect of the Cellophane is not sharply selective, all ultra-violet wave-lengths emitted by the lamp being transmitted, though with somewhat greater loss of intensity in the shorter than in the longer wave-lengths. The heating effect on the pollen was reduced (but not eliminated) by an electric fan ventilating the space between the arc and the Petri dish. Under these conditions treatments of 2 to 8 minutes could be made, but few seeds were produced by pollen irradiated for 16 minutes.

The effects of unfiltered radiation were determined from the results of a series of treatments made in 1934. The treated pollen was applied to ears of two stocks recessive for several genes affecting endosperm characters: (1) *a a wx wx su su pr pr y y* (here designated "*a*-recessive"), and (2) *c c wx wx su su pr pr y y* (designated "*c*-recessive"). Since the pollen stock used in this series of trials was *A A C C Wx Wx Su Su Pr pr Y y*, deficiencies affecting the entire endosperm could be detected only for the genes *A*, *C*, *Wx* and *Su*, but fractionals for *Pr* and *Y* could be detected in half of the seeds produced.

Endosperm Deficiencies.—The ears resulting from these pollinations showed a distinct increase in the frequency of deficiencies marked by the endosperm characters. In addition to deficiencies affecting the entire endosperm, many deficiencies affecting only part of the endosperm ("fractionals") were observed. Fractionals were classified, according to the approximate fraction of the surface showing the recessive character, as "$^{15}/_{16}$", "$^{7}/_{8}$", "$^{3}/_{4}$", "$^{1}/_{2}$", "$^{1}/_{4}$", "$^{1}/_{8}$", "$^{1}/_{16}$" and "small". Comparison with the control in this experiment, and with standard control values previously determined in large populations of the same stocks, showed no significant increase in the frequency of the smaller fractionals ("$^{1}/_{16}$" and "small"). There was a pronounced increase in the frequency of fractionals of the "$^{1}/_{2}$" class, and a smaller, though clearly significant, increase in the frequency of fractionals of other classes from "$^{7}/_{8}$" to "$^{1}/_{8}$", those larger than "$^{1}/_{2}$" being increased in frequency to about the same extent as those smaller. It seems probable, therefore, that the fractionals induced by ultra-violet treatment of pollen are in general the result of deficiency affecting the cell progeny of one of the two cells produced by the first division of the endosperm-fusion-nucleus.

The "*c*-recessive" stock included the markers *c* and *wx*, which are linked with about 26% crossing-over. Losses of *C* and *Wx* were usually associated,

indicating that losses were due in most cases, if not in all, to deficiency rather than to recessive mutation. With the other genes used it is assumed that the loss of the dominant marker was due similarly to deficiency, although there is no way of excluding the possibility that some of these losses were due to induced mutation.

The frequency of endosperm deficiencies involving *A*, *Su*, *Wx* and *Pr* is shown in table 1. Data on fractional deficiencies involving *Wx* and *Y* are omitted from the table, since, in seeds with colored aleurone, it is impossible to insure the detection of all fractionals for these characters.

Ultra-violet treatment of the pollen, like x-ray treatment, increases the frequency of endosperm deficiency. The number of deficiencies affecting the entire endosperm, with the ultra-violet doses used in this experiment, is considerably lower than the number resulting from heavy doses of x-rays. The effect of the ultra-violet treatment on the frequency of endosperm

TABLE 1

EFFECT OF UNFILTERED ULTRA-VIOLET RADIATION ON FREQUENCY OF ENDOSPERM DEFICIENCIES

	"*a*-RECESSIVE" STOCK								"*c*-RECESSIVE" STOCK							
	NO. SEEDS EXAMINED	DEFICIENCIES OBSERVED ENTIRE				FRACTIONAL				NO. SEEDS EXAMINED	DEFICIENCIES OBSERVED ENTIRE				FRACTIONAL			
TREATMENT		*A*	*Su*	*Wx*	TOTAL	*A*	*Su*	*Pr*	TOTAL		*C*	*Su*	*Wx*	TOTAL[1]	*C*	*Su*	*Pr*	TOTAL
2 min.	252	0	0	0	0	3	0	2	5	265	0	1	0	1	2	2	0	3
4 min.	704	4	2	0	6	19	11	12	42	881	0	5	0	5	5	4	1	10
8 min.	99	4	1	0	5	4	1	0	5	371	3	4	3	7	1	4	2	7
16 min.										90	1	0	1	1	3	0	0	3
Total	1055	8	3	0	11	26	12	14	52	1607	4	10	4	13	11	10	3	23
Control	1893	0	0	0	0	1	1	0	2	2543	0	2	1	3	0	3	1	4

[1] *Wx* omitted from total since loss of *C* and *Wx* is usually coincidental.

chimeras (fractionals) is in marked contrast to the effect of x-ray treatment of pollen at the same stage. As previously reported,[7,8] x-ray treatment of ears approximately at the time of fertilization greatly increases the frequency of endosperm chimeras, but x-ray treatment of pollen, though it causes a very large increase in the frequency of deficiencies affecting the entire endosperm, has little or no effect on the frequency of endosperm chimeras.

Deficiencies affecting the embryo instead of the endosperm may be identified, similarly, by the examination of the F_1 plants for plant characters dominant in the pollen parent and recessive in the seed parent. In this series of treatments, the only marker affecting a plant character was *A* in the crosses on the "*a*-recessive" stock, and no losses of *A* were found among the F_1 plants grown. In later trials with better-marked stocks and larger populations, deficiencies affecting plant characters have been found.

These furnish material for the cytological study of deficiency induced by ultra-violet treatment.

Gametophytic Lethals.—Independently of the use of marker genes, deficiencies induced by the treatment may be identified by their effect on pollen development in the F_1 plants. Deficiency at any locus, if it involves genes essential to the normal development of the haploid gametophyte, should cause the abortion or abnormal development of the deficient microspores and megaspores. Although it has been shown that some deficiencies in maize may permit apparently normal functioning of the female gametophyte,[9] no deficiency is known which permits normal development of the pollen. In plants heterozygous for a deficiency half of the pollen is defective, the degree of defectiveness being characteristic of the deficiency concerned. Induced deficiencies, therefore, may be identified *en masse* by examination of the pollen of the F_1 plants.

Segregations for defective pollen may result from genetic alterations other than deficiency. In addition to possible gene mutations affecting pollen development (which cannot be distinguished from short deficiencies), there are certain chromosomal derangements which result in partial pollen abortion. Heterozygous translocations usually have approximately 50 % defective pollen, and heterozygous inversions have defective pollen in proportions determined by the frequency of crossing-over in the inverted segment. Translocations occur frequently as a result of x-ray treatment of pollen, and inversions, though relatively infrequent, are not rare. By analogy with x-ray treatment, it would be expected that the chromosomal derangements identified by the occurrence of defective pollen in the F_1 plants would be made up chiefly of deficiencies and translocations. The frequency of deficiency determined in this way is a minimal value, since only the plants reaching flowering are examined, and deficient plants are more likely to be lost before flowering than non-deficient plants. Plants heterozygous for translocations are normal in development, and their frequency should not be affected by differential mortality.

Deficiencies may be distinguished from translocations by testing transmission of the genetic complex resulting in defective pollen. Translocations are regularly transmitted through both male and female germ cells, while deficiencies producing defective pollen are not transmitted at all through male germ cells, and are transmitted through female germ cells only in exceptional cases (haplo-viable deficiencies). In these trials, since it was desirable to identify and maintain the haplo-viable deficiencies, transmission through female germ cells was determined, and in cases showing transmission a cytological examination of heterozygous plants was made at diakinesis to distinguish between deficiency and translocation.

Approximately three-fourths of the seed produced by the use of treated pollen and about one-fifth of the seed from the control ears were planted,

and in all plants which reached the flowering stage, pollen was examined for defective pollen segregations. Thirty-eight segregating plants were found among the 1065 plants from treated pollen examined, and 3 segregating plants among 632 control plants examined. Each segregating plant was back-crossed with the multiple recessive parent and in most cases also was self-fertilized. In several cases the ratios of the marker genes used indicated a deficiency affecting a specific chromosome. In one instance a close association of sugary endosperm and aleurone color in the back-cross progeny indicated a translocation involving chromosome 4 and chromosome 9 or 10.

Preliminary tests of transmission of the defective-pollen complex through female germ cells have been made in 29 of the 38 cases. Transmission was found in 15 cases. In each of these, cytological examination of segregating plants at diakinesis showed 10 bivalents. The absence of rings or chains involving two or more pairs of chromosomes indicates that translocation was not responsible for the defective pollen in any of these cases. Thus, except for the genetic indication of translocation in one F_1 plant (not yet confirmed cytologically), there was no indication of any effect of ultra-violet treatment of pollen on the frequency of translocation. The frequency of haplo-viable deficiencies indicated by the proportion of pollen defects transmitted is surprisingly high.

Mutations.—All of the F_1 plants were self-fertilized and the resulting F_2 ears examined for the occurrence of mutations affecting seed characters. A sample of 25 seeds was planted from each ear and the resulting seedling progenies examined for mutations affecting seedling characters. Among 830 F_2 ears from treated pollen, 31 recessive mutations were found, including 19 affecting seed characters and 12 affecting seedling characters. Among the 557 F_2 ears of the control, 6 recessive mutations were found, 3 affecting the seed and 3 the seedling. The mutations affecting seed characters included a variety of defective types, such as "small," "scarred," "rudimentary," "miniature," "aborted," "germless" and "gnarled," and one distinctive new aleurone variant designated "aleurone-spot." The seedling mutations included "white," "virescent," "yellow-green," "speckled," "glossy," "rolled," "dwarf" and "corrugated." Many of these mutants resemble recessives previously known, but no tests of genetic identity have yet been made.

A striking feature of the mutation results was the occurrence of three cases of association of two or more mutants in a single F_2 progeny, indicating the occurrence of two or more mutations in the same treated sperm. One F_2 ear showed segregation of both miniature seeds and glossy seedlings, another germless seeds and yellow-green seedlings, a third rudimentary seeds and both yellow-green and virescent-white seedlings. In each case the mutants were unlinked, or at any rate not linked closely enough to show

significant association in populations of 100 to 300 individuals. The endosperm mutant "aleurone-spot" and the seedling mutant "corrugated" occurred in the same F_2 progeny, but showed complete linkage in the population grown, and therefore may represent two effects of the same mutant gene. In addition, three of the mutants (small seed, miniature seed, white-tip seedling) were found in progenies of F_1 plants segregating for defective pollen.

The number of cases of apparently independent genetic variations occurring in the same treated gamete is much higher than that expected from chance coincidence. But not enough is known as yet of the absorption of ultra-violet radiation in pollen to make possible even a rough estimate of the proportion of the incident energy which reaches the sperm nucleus. It is possible that the sperm nuclei receive an effective dose only in the most favorably oriented pollen grains. If so, coincidences would be expected in larger numbers. However, if this is the explanation of the coincidences observed, the frequency of induced mutation in effectively treated nuclei must be extremely high.

Summary.—1. Ultra-violet radiation applied to the pollen of maize greatly increased the frequency of both entire and fractional endosperm deficiencies. The average size of the fraction showing deficiency was approximately one-half of the endosperm.

2. Deficiencies affecting the F_1 plants also were induced by the treatment. Many of these were haplo-viable.

3. There was no significant increase in the frequency of translocation.

4. Numerous point mutations affecting seed and seedling characters were induced by the treatment. Several of these occurred in treated germ cells in which apparently unrelated mutations or other germinal alterations also occurred.

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GENETIC EFFECTS OF ULTRA-VIOLET RADIATION IN MAIZE. II. FILTERED RADIATIONS

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In treatments with filtered radiations the commercial arc described in the preceding paper was used in combination with various solution filters. In order to minimize heating effects and to increase the intensity of the radiation applied, a water cooling system was provided and the treating distance was reduced to 6.8 cm. The pollen, lying in a single layer on a glass slide, was placed in a brass "treatment cell" cooled by cold water circulating within its hollow walls. The treatment cell was covered by the "filter cell," a rectangular box of fused quartz, divided horizontally into two compartments each 1 cm. high. The lower compartment contained circulating distilled water for additional cooling and the upper contained the filter solution. The luminous portion of the arc was directly above the filter cell. Thus the radiation in all cases was filtered through 1 cm. of distilled water and about 3 mm. of fused quartz in addition to the solution filter used.

Preliminary trials with filtered radiations were made with a series of "long wave-pass" liquid filters, designed to detect gross differences in the effectiveness of broad spectral bands between 2500 and 3300 Å. Each of these filters absorbs all, or nearly all, radiation of wave-length shorter than a given value *a*, and passes with little absorption radiation of wave-length longer than a second value *b*, with a relatively narrow band of sharply decreasing absorption between *a* and *b*. The transmission characteristics of these filters and of the additional $HgCl_2$ filters mentioned below were determined by E. W. Landen, who will describe them in a separate report.

These trials were made with a multiple dominant stock and the "*a*-recessive" and "*c*-recessive" stocks described in the preceding paper. The results indicated that effectiveness in inducing endosperm deficiencies at all of the loci tested decreases sharply in the spectral region about 3000 Å. Numerous endosperm deficiencies occurred following treatment with radiation filtered through a solution of $HgCl_2$ which reduced sharply the intensity of λ 2804 and shorter wave-lengths but reduced only slightly the intensity of λ 2925 and longer wave-lengths. No endosperm deficiencies occurred (in populations of moderate size) following treatment with radiation filtered through a stronger solution of $HgCl_2$ which reduced sharply the intensity of λ 2967 and shorter wave-lengths while reducing only slightly that of λ 3130 and longer wave-lengths. This result showed also that the

deficiencies induced by the effective treatments could not have been due to temperature effects, for the temperature of the pollen could not have differed materially in the treatments with these two filtered radiations.

More extensive trials were then made with three $HgCl_2$ solution filters, two similar to those used in the preliminary trial and the other intermediate in absorption. The filtered radiations are referred to below as radiations *A*, *B* and *C*. The spectral distribution of energy in the three radiations was determined by passing each radiation through a spectrograph and measuring the relative intensity line by line by means of a quartz-enclosed caesium oxide photocell of the type described by Young and Pierce.[1] The concentration of the filter solutions and the spectral distribution of energy in the filtered radiations are shown in table 1.

Treatments were made with the three filtered radiations with exposure periods of 1 to 32 minutes. The length of treatment tolerated by the pollen varies widely under different conditions of temperature and humidity, but under similar conditions there were consistent differences in tolerance of the three radiations. The maximum period of exposure tolerated with radiation *C* was ordinarily 4 to 8 times as long as that with radiation *A*, and was not consistently shorter than the period of delay in pollination tolerated by untreated pollen under similar conditions. Tolerance of radiation *B* was only slightly less than that of radiation *C*.

The endosperm constitution of the pollen parent in these trials was *A A Pr Pr;* that of the seed parent *a a Pr pr*. The results therefore show the frequency of loss of *A* and one-half the frequency of loss of *Pr*. The frequency of germless seeds under the three treatments was also determined. The results are presented in table 2.

The frequency of entire endosperm deficiencies for *A* and *Pr* is approximately equal, while the frequency of fractional deficiencies is very much higher for *A* than for *Pr*. Both entire and fractional endosperm deficiencies are materially increased in frequency by the treatment of the pollen. Radiation *A* is distinctly more effective than *C*, as measured by the frequency of entire or fractional endosperm deficiencies for either *A* or *Pr*. Radiation *B* is intermediate in effectiveness.

The frequency of germless seeds is increased slightly by treatment with radiation *A*, distinctly less by radiation *B* and not at all by radiation *C*. With x-ray treatment of pollen the frequency of germless seeds is much higher, and is usually considerably higher than the frequency of deficiencies marked by any one gene.

In comparing the three radiations, it is convenient to use a single figure expressing the effectiveness of the radiation in producing both entire and fractional deficiencies of both *A* and *Pr*. The measure used is computed as follows: From the numbers of entire and fractional deficiencies for *A* and *Pr* observed under each treatment are deducted the numbers expected

in control populations of the same size. The remaining deficiencies, presumably those induced by the treatment, are combined by adding the total number of entire deficiencies and half the total number of fractionals. In this trial, as in that previously reported, the induced fractionals were chiefly of the "1/2" class, with about equal numbers of larger and smaller size. Thus calculated, the yield of induced deficiencies per minute of exposure for filtered radiations *A*, *B* and *C* is 1.99, 1.25 and 0.24%, respectively. These percentages represent only a small part of the deficien-

TABLE 1

SPECTRAL DISTRIBUTION OF ENERGY IN FILTERED RADIATIONS
(Data of E. W. Landen)

WAVE LENGTH Å	UNFILTERED	FILTERED RADIATIONS[1] A	B	C
3654	105.5	74.7	74.4	74.1
3341	10.5	6.95	7.13	6.79
3130	100	61.9	58.6	53.5
3022	44.1	25.2	21.7	9.87
2967	22.7	11.4	7.88	0.772
2925	2.22	1.04	0.420	0.004
2894	6.90	2.48	0.470	..
2804	11.0	0.465	.0033	..
2752	3.08	0.017	..	..
2699	3.47	..	..	..
2652	15.72	..	..	..
2602	2.07	..	..	..
2576	2.06	..	..	..
2537	24.8	..	..	..
2482	3.90	..	..	..
2463	0.60	..	..	..
2447	0.54	..	..	..
2399	1.47	..	..	..
2378	1.28	..	..	..
2352	0.81	..	..	..
2323	0.43	..	..	..
2300	0.63	..	..	..

[1] Radiations were filtered through 1 cm. of distilled water and 3 mm. of fused quartz plus 1 cm. of filter solution as follows: (A) 30 g. $HgCl_2$ + 15.4 cc. HCl per liter, (B) 90 g. $HgCl_2$ + 46.2 cc. HCl per liter and (C) 270 g. $HgCl_2$ + 140 cc. HCl per liter. Energy measurements are in arbitrary units.

cies induced by the treatment, since only the deficiencies marked by one gene and half the deficiencies marked by another gene are included. They are, however, strictly comparable for the radiations compared.

Since the filtered radiations differ so widely in effectiveness, it is clear that λ 3130 and longer wave-lengths are relatively ineffective, for the energy in this region of the spectrum was relatively high in radiation *C* as well as in radiations *A* and *B*. Such differences as are found must be

ascribable in the main to differences in the intensity of the shorter wave-lengths. If all wave-lengths shorter than λ 3130 are equally effective, and λ 3130 and longer wave-lengths are wholly ineffective, the total effective energy in radiations *A*, *B* and *C* is approximately in the ratio 4:3:1. This is a considerable departure from the observed ratio of induced deficiencies, namely, 8:5:1. The fit may be materially improved by ascribing reduced effectiveness to λ 3022. For example, on the assumption that λ 3022 is only one-sixth as effective as shorter wave-lengths, the ratio is

TABLE 2

EFFECT OF VARIOUS FILTERED ULTRA-VIOLET RADIATIONS ON FREQUENCY OF ENDOSPERM DEFICIENCIES

$HgCl_2$ FILTER	TIME, MIN.	NUMBER SEEDS EXAMINED	DEFICIENCIES OBSERVED: ENTIRE A	ENTIRE PR	ENTIRE TOTAL	ENTIRE %	FRACTIONAL A	FRACTIONAL PR	FRACTIONAL TOTAL	FRACTIONAL %	NUMBER GERMLESS SEEDS
A (30 g./l.)	1	797	6	2	8	1.0	22	1	23	2.9	11
	2	1247	18	13	31	2.5	73	9	81	6.5	9
	4	717	20	15	35	4.9	46	11	57	7.9	13
	8	218	10	1	11		18	3	21		8
	16	28	1	0	1		1	0	1		4
Mean yield per min. exposure:						1.03				2.20	
B (90 g./l.)	2	1614	29	15	44	2.7	50	7	57	3.5	7
	4	1100	24	10	34	3.1	50	11	61	5.5	2
	8	861	27	7	34	3.9	65	10	75	8.7	10
	16	65	1	0	1		7	1	8		2
	32	16	0	1	1		1	0	1		1
Mean yield per min. exposure:						0.71				1.26	
C (270 g./l.)	2	950	0	0	0	0.0	5	1	6	0.6	1
	4	1620	6	5	11	0.7	24	6	30	1.9	0
	8	903	6	3	9	1.0	15	2	17	1.9	5
	16	848	12	5	17	2.0	43	7	50	5.9	0
	32	128	2	2	4		6	2	8		4
Mean yield per min. exposure:						0.12				0.33	
Control	..	1941	1	2	3	0.15	7	2	9	0.46	5

modified to 8:5:1. If, on the other hand, it is assumed that λ 3022 is entirely ineffective, the ratio of effective energy is approximately 20:11:1; and on the plausible assumption that λ 2804 and shorter wave-lengths are more effective than λ 2967, the fit becomes even poorer. It is therefore necessary to ascribe some effectiveness to λ 3022, although it is probable that its effectiveness is lower than that of shorter wave-lengths.

The indicated differences in the effectiveness of various wave-lengths are not necessarily due to differences in the reaction of the cromosomes to the wave-lengths concerned. They are due in part, possibly in large part, to differences in the penetration of the different wave-lengths through the overlying non-nuclear material. The sperm nucleus, within which the

chromosomal alterations are induced, is embedded in a mass of cytoplasm and cytoplasmic inclusions which consitutes the bulk of the pollen grain. The spectral distribution of energy in the radiations applied, as shown in table 1, represents the composition of the radiation incident at the surface of the pollen grain. The spectral constitution of the radiation reaching the sperm nucleus may be very different. Until the absorption characteristics of the various constituents of the pollen grain are more definitely known, no accurate comparison of the direct effects of different wave-lengths upon the chromosomes can be made.

The apparent effectiveness of λ 3022 and λ 2967 is of interest from an evolutionary viewpoint, since wave-lengths in this region are sometimes included in low intensity in summer sunlight. Under natural conditions the pollen grain may be exposed to sunlight for a brief period just preceding and just following pollination. Genetic changes induced by the ultra-violet constituent of sunlight would be included in the "natural" mutation rate. Data are not available for estimating the total intensity of radiations of genetically effective wave-length to which the pollen may be exposed under natural conditions of pollination; nor is the natural frequency of endosperm deficiencies, gametophytic lethals and mutations under conditions of open pollination accurately known. However, the controls in these experiments, which yielded several endosperm deficiencies, gametophytic lethals and mutations, were produced by the use of pollen shed indoors and shielded from sunlight before and after pollination. It is clear, therefore, that exposure to sunlight at this period is not the sole cause of naturally occurring deficiencies and mutations.

Summary.—1. Results with filtered radiations applied to maize pollen indicate that λ 3130 and longer wave-lengths are relatively ineffective in inducing deficiency, and that λ 3022 and shorter wave-lengths are effective. λ 3022 is probably less effective than shorter wave-lengths.

2. Although radiation of effective wave-length is sometimes present in low intensity in sunlight, the occurrence of various genetic alterations in the controls (shielded from sunlight) shows that exposure of the pollen to sunlight is not the sole cause of naturally occurring deficiencies and mutations.

3. The frequency of entire endosperm deficiencies of *A* and *Pr* induced by the ultra-violet radiations applied is approximately equal. The frequency of induced fractional endosperm deficiencies is much higher for *A* than for *Pr*.

4. The frequency of germless seeds induced by the ultra-violet radiations applied is low, and decreases with decreasing relative intensity of the shorter wave-lengths.

[1] Young and Pierce, *Jour. Opt. Soc. Am.*, **21**, 497–501 (1931).

GENETIC EFFECTS OF ULTRA-VIOLET RADIATION IN MAIZE. III. EFFECTS OF NEARLY MONOCHROMATIC λ 2537, AND COMPARISON OF EFFECTS OF X-RAY AND ULTRA-VIOLET TREATMENT

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A high voltage mercury discharge tube of fused quartz emits radiation consisting chiefly of λ 2537. A commercial tube of this sort (Hanovia (Sc-2537), operated at 5000 volts and 100 m. a., was used as a source of radiation in comparison with filtered radiations of the types described in the preceding paper.

The discharge tube treatments were applied under conditions similar to those used with the mercury arc. Exposures were made at the same distance and with the same filter cell, with 1 cm. of distilled water in place of the filters used with the mercury arc. Under these conditions about 83% of the total intensity is λ 2537. Other wave-lengths included are chiefly in the near ultra-violet and visible. Of the wave-lengths shorter than λ 3130, more than 97% of the total intensity is λ 2537. For the purposes of genetic experiments, therefore, this radiation is almost monochromatic λ 2537. The ratio of intensity of the radiation from the discharge tube and the arc (the water filter being used in both cases) was for λ 2537 approximately 1:1, for total radiation shorter than λ 3130 approximately 1:8.

In experiments with the discharge tube, the multiple recessive seed parent used was *A a C c wx wx su su pr pr y y*. The pollen stock carried the dominant alleles of all of these genes. It was therefore possible to detect one-half of the losses of *A* and *C* (as indistinguishable color losses) and all of the losses of *Wx*, *Su*, *Pr* and *Y*.

The maximum dose tolerated is considerably lower than the maximum tolerated dose of the filtered radiations reported above. Only the most approximate comparisons may be made in tolerance because of the variation with environmental conditions which has been mentioned. In treatments with the discharge tube, under favorable weather conditions and with excess pollen, well-filled ears are produced by the use of pollen irradiated for 30 seconds or less, but only partial seed setting occurs with pollen irradiated 60 seconds or more. A few seeds are produced by the use of pollen treated as long as 120 seconds. Under similar conditions, with filtered radiation *A* of the mercury arc (described in the preceding paper), well-filled ears are produced by the use of pollen irradiated 2 minutes and sometimes 4 minutes, and a few seeds are produced by pollen treated as

long as 16 minutes. The total intensity of ultra-violet radiation of wave-lengths shorter than λ 3130 is considerably higher in radiation *A* than in the discharge tube radiation, but about 90% of it is λ 2967 and λ 3022. When the mercury arc is used with the water filter, the maximum exposures tolerated are only about half as long as with the discharge tube. These observations indicate that the wave-lengths which must be chiefly responsible for the genetic effects of filtered radiations *A* and *B* (λ 2967 and λ 3022) have much less "killing" effect than λ 2537 and other short wave-lengths.

TABLE 1

COMPARATIVE EFFECT OF VARIOUS ULTRA-VIOLET RADIATIONS ON FREQUENCY OF ENDOSPERM DEFICIENCIES AND GERMLESS SEEDS

	DISCHARGE TUBE WATER FILTER	MERCURY ARC FILTER A	MERCURY ARC FILTER B	CONTROL
No. Seeds Examined	1199	695	525	578
Mean Exposure Period[1] (Sec.)	30.8	128.4	217.8	....
Deficiencies				
Entire (E)				
$\frac{A+C}{2}$	17	8	4	0
Pr	13	10	5	1
Su	10	7	4	0
Y	6	2	4	2
Fractional (F)				
$\frac{A+C}{2}$	28	23	8	2
Pr	8	7	7	0
Su	10	9	7	0
Y	6	2	5	0
Total $E+\frac{F}{2}$	72	47.5	30.5	4
%	6.0	6.8	5.8	0.7
Germless Seeds	62	7	4	2
%	5.2	1.0	0.8	0.3

[1] Weighted mean of varying periods of exposure.

The effect of the water-filtered radiation from the discharge tube upon the frequency of endosperm deficiencies and germless seeds, together with comparable results of filtered radiations of the mercury arc, are shown in table 1. *Wx* deficiencies are omitted from this table, since the seeds affected are in some cases included among the *C* deficiencies observed.

With each radiation the treatments applied included a rather wide range of dosage. The relation of dosage to genetic effectiveness cannot be determined accurately with the mercury arc because of uncontrolled fluctuations in intensity with temperature and other conditions. The discharge tube radiation is less variable. In treatments with the discharge

tube the doses ranged from $7^1/_2$ to 120 seconds, and the full dosage series was carried out in consecutive treatments during a two-hour period, with a complete repetition the following morning, using the same plant as pollen source and maintaining conditions as uniform as possible. The results are subject to a rather high probable error due to the small populations available, but they indicate that the relation of total frequency of deficiency to dosage is approximately linear. In table 1 the results at all doses used are summarized for each radiation, the weighted mean of exposure periods being given to permit comparison of the effect of the different radiations in equivalent exposures.

The relative frequency of loss of the various marker genes used does not differ materially in the trials with the discharge tube radiation and the various filtered radiations. In both the number of deficiencies is somewhat lower for *Y* than for the other genes tested, and the frequency of fractionals is distinctly higher for the aleurone color genes *A* and *C* than for the other markers. Unfortunately, the effects due to *A* and *C* losses cannot be separated in this mating. The results with *A* in the previous trials suggest that the preponderances of fractionals for aleurone color in this trial may be due chiefly to *A*. This is supported by the fact that only a small proportion of the fractional color losses reported in table 1 involve *Wx*, although trials with *c wx* stocks (with unfiltered ultra-violet radiation) had indicated that most *C* losses involve *Wx* also.

Although the relative frequency of entire and fractional losses apparently differs at different loci, the frequency of fractional deficiencies is clearly increased at all loci by the various ultra-violet treatments. This extends to additional loci the contrast previously noted between ultra-violet and x-ray treatment of the pollen in their effect on the frequency of fractional deficienes.

The genetic effect of the low doses tolerated with the discharge tube radiation was approximately equal to that of the relatively high doses of the filtered radiations. Considering the observed genetic effects in relation to the spectral distribution of energy in the radiations applied, it is evident that λ 2537 is much more effective than either λ 2967 or λ 3022 per unit of energy applied at the surface of the pollen grain.

The most striking difference in the trials of the relatively short wave-length radiation of the discharge tube and the longer wave-length filtered radiations is in their effect on the frequency of germless seeds. Comparing doses approximately equal in frequency of induced endosperm deficiencies, the percentage of germless seeds in excess of the control frequency was about nine times as large for the discharge tube radiation as for the filtered radiations. The difference is consistent in the various pollinations summarized in table 1, and is confirmed by additional comparisons in other matings. This indicates that the physical alterations in the treated pollen

which result in the production of germless seeds are essentially different from the alterations which result in induced deficiencies, and are relatively much less affected by the longer wave-lengths. The comparison of discharge tube and filtered radiations thus shows that much smaller doses of λ 2537 than of λ 2967 and λ 3022 are required for a given genetic effect, and that even with these smaller doses the shorter wave-length has a much more pronounced effect on the frequency of germless seeds.

The reduced tolerance to treatments with the shorter wave-lengths may be due in part to effects analogous to those resulting in germless seeds. The type of alteration in the sperm which results in failure of the embryo to develop, may, when it affects the other sperm, sometimes result in failure of development of the endosperm and thus of the seed as a whole. Such alterations would reduce the proportion of seed set, regardless of the presence of excess pollen, while changes affecting germination of the pollen grain, growth rate of the pollen tube, etc., would not affect the set if the amount of pollen not so affected were sufficient for fertilization of all of the ovules.

Comparison of the Effects of Ultra-Violet and X-ray Treatment.—The effects of ultra-violet treatment in these experiments have differed in various ways from the effects previously noted in similar experiments with x-ray treatment of pollen. The contrasts indicated were the following:

(1) Among the endosperm deficiencies induced, the proportion of fractionals is much higher with ultra-violet treatment than with x-rays.

(2) The proportion of germless seeds produced by ultra-violet treated pollen is much lower than that produced by x-rayed pollen. With the longest ultra-violet wave-lengths effective in inducing deficiency, the effect on frequency of germless seeds is almost inappreciable.

(3) There is no evidence of any increase in frequency of translocation under ultra-violet treatment.

(4) Among deficiencies affecting the F_1 plants, the proportion of haplo-viable deficiencies may be higher with ultra-violet than with x-ray treatment.

(5) Of the mutations induced by ultra-violet treatment, several occurred in germ cells in which apparently unrelated mutations or other germinal alterations also occurred.

All of these apparent contrasts are based on comparison of the results of experiments made at different times and with different stocks. Obviously they must be confirmed in strictly comparable trials before they may be considered conclusive. Such trials are now being made with various ultra-violet treatments in comparison with x-ray treatments covering a wide range of dosage. The range in dosage is necessary in order to distinguish between differences in effect due to spectral variations in activity and those which may be incidental to dosage differences. For example, if

translocations are rare following ultra-violet treatment and common following x-ray treatment, it does not follow necessarily that translocations are dependent upon some initial physical change induced by x-rays and not by ultra-violet. An alternative possibility is that translocations occur by deferred reattachment following chromosome breakage, as suggested by Stadler[1] and since the number of chromosome breaks is much smaller in the ultra-violet treated cells, the opportunities for interchange in reattachment are extremely rare. If so, x-ray doses producing the same number of chromosome breaks as the ultra-violet treatments used also should fail to increase appreciably the frequency of translocation.

Comparable data for x-ray and ultra-violet treatment are now available only for the effect on frequency of endosperm deficiencies and germless seeds. In connection with the trials of filtered radiations reported in the preceding paper, comparable pollinations were made with x-rayed pollen. The freshly dehisced anthers were treated in a shell vial just before pollination. The radiation was that emitted by a Coolidge tube operated at 140 K.V.P., with no filtration except that of the glass vial. The dose was 1333 r.

In comparing the effects of x-ray and ultra-violet treatment on the frequency of entire and fractional endosperm deficiencies, the deficient endosperms showing "recovery" are a source of some difficulty. "Recovery" is the term applied to the occurrence of small islands of non-deficient tissues in individuals otherwise deficient. Among the deficiencies induced by x-ray treatment, a small fraction, varying with the marker used, show recovery. For reasons previously discussed[1,2] these are considered a group distinct from the endosperm mosaics, and are ascribed to segmental deficiency affecting the endosperm as a whole followed by recovery of the segment in one or more cells in the course of endosperm development. In some instances, however, it is impossible to distinguish between recovery and mosaic individuals. If the development of the endosperm were a geometrically regular process, all mosaics due to loss occurring in the course of endosperm development would show the recessive character in sector of $^1/_2$, $^1/_4$, $^1/_8$, $^1/_{16}$, ... , depending on the cell generation in which the loss occurred, while recovery would be manifested by endosperms showing the recessive character in $^1/_2$, $^3/_4$, $^7/_8$, $^{15}/_{16}$, ... , depending on the cell generation in which the segment was restored to normal distribution and activity. But endosperm development is extremely irregular and consequently it is impossible to determine in individual cases the cell generation in which a loss or recovery occurred. In practice we draw an arbitrary line between the two groups, classifying the entire series $^{15}/_{16}$ to $^1/_{16}$ as fractionals and classifying definitely as deficiencies followed by recovery only those seeds in which the total fraction showing the dominant character is estimated as $^1/_{32}$ or less. Many of the seeds classified as frac-

tionals with large recessive sectors may be the result of the same phenomenon as those in the recovery class. In comparing the effects of x-ray and ultra-violet treatments, which appear to differ in their relative effect on entire and fractional deficiencies, it is therefore desirable to distinguish between fractionals of the various classes. In classifying the seeds, the size of the recessive sector was carefully estimated in each fractional ex-

TABLE 2

COMPARATIVE EFFECT OF X-RAYS AND ULTRA-VIOLET RADIATION ON ENDOSPERM DEFICIENCIES AND GERMLESS SEEDS

	X-RAYED POLLEN (1333 r)	ULTRA-VIOLET-TREATED POLLEN FILTER A	FILTER B	FILTER C	CONTROL
No. Seeds Examined	1901	3007	3656	4449	1941
Mean Exposure Period (Min.)	4.55	2.78	4.39	7.48	.....
Deficiencies					
A. Entire					
(1) Without Recovery					
A	128	50	75	23	1
Pr	20	31	33	15	2
Total (%)	7.79	2.69	2.95	0.85	0.15
(2) With Recovery ($^{31}/_{32}$ or more of recessive tissue)					
A	38	5	6	3	0
Pr	0	0	0	0	0
Total (%)	2.00	0.17	0.16	0.07	0.00
B. Fractional					
(3) $^{15}/_{16}$–$^{7}/_{8}$ recessive tissue					
A	23	16	22	9	1
Pr	0	0	1	0	0
Total (%)	1.21	0.53	0.63	0.20	0.05
(4) $^{3}/_{4}$–$^{1}/_{4}$ recessive tissue					
A	14	116	125	74	4
Pr	1	21	26	16	1
Total (%)	0.79	4.56	4.13	2.02	0.26
(5) $^{1}/_{8}$–$^{1}/_{16}$ recessive tissue					
A	14	28	26	10	2
Pr	2	3	2	2	1
Total (%)	0.84	1.03	0.77	0.27	0.15
Germless Seeds	340	45	22	10	5
%	17.89	1.50	0.60	0.22	0.26

amined. In table 2 the data on endosperm deficiencies are summarized in five classes, based on the extent of the tissue showing the recessive character.

The contrast in effect of the two kinds of radiation on fractional and entire endosperm deficiencies is clearly shown by these data. Comparing x-ray treatment with ultra-violet radiation *A*, for example, the dose of 1333 r produces about three times as high a percentage of entire endosperm deficiencies as the mean ultra-violet dose used, but (even if all deficiencies with recovery be included among the fractionals) a distinctly smaller

percentage of fractionals. The fractionals following ultra-violet treatment are symmetrically distributed about the $^1/_2$ class, but those following x-ray treatment are most frequent in group 3 ($^{15}/_{16}$–$^7/_8$) with a still larger number in the recovery class (group 2), corresponding to $^{31}/_{32}$ or a higher fraction of the endosperm recessive. This suggests that most of the apparent fractionals induced by x-ray treatment are in fact due to recovery, and that this treatment has little effect on the phenomenon responsible for fractionals of the type induced by ultra-violet treatment.

It is clear also that the relative effect of the two types of radiation on deficiencies at the two loci marked is widely different. As previously stated, the *Pr* deficiencies noted represent the frequency of deficiency for only half of the population, since the seed parent was heterozygous *Pr pr*. For "entire" endosperm effects (groups 1 and 2) the percentage of *A* and *Pr* deficiencies respectively under x-ray treatment was 8.73 and 2.10; under ultra-violet treatment *A*, 1.83 and 2.06. Results with the other ultra-violet radiations were similar, the total frequency of entire deficiencies of *Pr* being about equal to that of entire deficiencies of *A* under ultra-violet treatment.

Finally, the frequency of germless seeds produced by x-ray treatment of pollen is very much higher than that produced by ultra-violet treatment. In this connection it should be recalled that ultra-violet radiations of shorter wave-length produce a much higher proportion of germless seeds than the filtered radiations here reported (see table 1), but the percentage of germless seeds produced by x-ray treatment is higher than that produced by any ultra-violet radiation used in these experiments.

Summary.—Comparable trials of mercury discharge tube radiation (largely λ 2537) and filtered mercury arc radiations (largely λ 2967 and λ 3022 plus various genetically ineffective wave-lengths) indicated:

(1) The relative frequency of induced deficiency at the various loci tested does not differ appreciably for the longer and shorter wave-lengths.

(2) The maximum dose tolerated is much lower for the shorter wave-length.

(3) The frequency of induced deficiency per unit of energy applied at the surface of the pollen grain is much higher for the shorter wave-length.

(4) The difference between the wave-lengths compared in effect on the frequency of germless seeds is even more extreme. Comparing doses which induced deficiency in approximately equal frequencies, the shorter wave-length radiation produced several times as many germless seeds as the longer wave-length radiation.

Comparable trials of x-rays (1333 r) and filtered mercury arc radiations (various doses) indicated:

(5) Almost all x-ray-induced deficiencies affect the endosperm as a

whole, while a large proportion of ultra-violet-induced deficiencies are fractionals affecting approximately half of the endosperm.

(6) The frequency of entire endosperm deficiencies of *A* is much higher than that of *Pr* following x-ray treatment, but *A* and *Pr* deficiencies are about equally frequent following ultra-violet treatment.

(7) The frequency of germless seeds produced by x-rayed pollen was much higher than that produced by ultra-violet-treated pollen.

Additional contrasts between ultra-violet and x-ray effects indicated by these experiments but not yet tested in strictly comparable trials are listed in the text.

[1] Stadler, *Proc. 6th Int. Cong. Genetics*, **1**, 274–294 (1932).
[2] Stadler, these PROCEEDINGS, **16**, 714–720 (1930).

A TERMINAL INVERSION IN DROSOPHILA ANANASSAE

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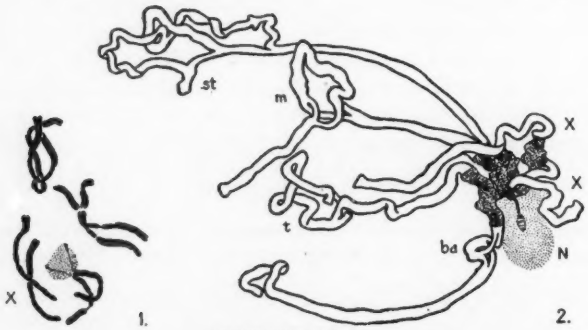
The existence of inverted segments in chromosomes of wild-type populations of *Drosophila melanogaster* was determined genetically by Sturtevant[1] from the effects which such inversions have in reducing crossing-over. Following the more recent application of the salivary gland method for cytological analysis of chromosome aberrations, naturally occurring inversions have been identified in several species of *Drosophila*. Thus, Tan[2] and Koller[3] found that race *A* and race *B* of *D. pseudoöbscura* differ in four inverted sections. Sturtevant and Dobzhansky[4] discovered inversions in wild populations of either race of *D. pseudoöbscura* inhabiting different geographical regions. Dubinin, Sokolov and Tiniakov[5] found a number of inversions widely distributed in different populations of *D. melanogaster* and *D. funebris*. Frolova[6] observed inversions in the chromosomes of *D. repleta* and *D. sulcata*. In the present study four inversions, one of which involves a terminal section of a chromosome, were found in *D. ananassae*. All four occur in autosomes.

The somatic chromosome complement of *D. ananassae* De Meijere (*D. caribbea* Sturtevant) has been determined by examination of the large cells of the ganglia of the larvae.[7] There are three pairs of *V*-shaped autosomes and a pair of *V*-shaped *X*-chromosomes in the female (Fig. 1). In the male the sex chromosomes are an *X* and a *J*-shaped *Y*. In prophase cells of female larvae, the nucleolus is associated with the pair of shortest autosomes, and separates a small chromomere-like satellite from the bulk

of the longer arm of these chromosomes. In males the Y-chromosome also is associated with the nucleolus.

In salivary gland nuclei of female larvae only six chromosome strands radiate from the chromocenter[8] instead of the eight which might be expected to result from conjugation of the four pairs of V-shaped homologs. Two of the six strands are relatively short, and represent the arms of the X-chromosomes, as has been determined by comparison with salivary gland nuclei of male larvae, in which these strands exist in the slender haploid condition. The other four strands represent the paired arms of the four longer autosomes. The shortest pair of autosomes exist in these salivary



FIGURES 1 AND 2

FIGURE 1. Prophase chromosomes, ganglion cell of female larva of D. ananassae. The nucleolus lies in contact with the shortest autosomes. X ca 2300.

FIGURE 2. Salivary gland chromosomes of female hybrid between two populations of D. ananassae. X—Limbs of paired X-chromosomes; N—Nucleolus with attached satellite; ba, m, st, t refer to basal, median, subterminal and terminal inversions in arms of paired autosomes. X ca 500.

gland nuclei solely as a small bipartite mass of heterochromatic material which forms a part of the chromocenter.[9] Evidence supporting this conclusion is obtained from the position of the nucleolus and the satellite, which maintain contact with this portion of the chromocenter region. This may be shown most strikingly in those preparations in which the pressure used in smearing the glands causes separation of the chromocenter into the component regions belonging to the contributing chromosomes. Details concerning nucleolus formation and the structure of the satellite will be presented in another publication.

The inversions were studied in hybrids obtained between individuals of the Alabama and a Japanese population.[7] Such individuals cross readily and produce fertile offspring. In salivary gland nuclei of all such

hybrids two inversions were seen, one of which is terminal (*t* in Fig. 2), the other basal in the other limb of the same chromosome pair (*ba* of Fig. 2). In a few salivary nuclei two other inversions were encountered in the second pair of longer autosomes, a subterminal one (*st*) in one arm, and a median one (*m*) in the other limb. The occurrence of these inversions in only a small proportion of the cells studied suggests that they are carried in a heterozygous condition in either the Alabama or Japanese material, but they have not been seen in salivary nuclei of these two stocks.

Special interest attaches to the terminal inversion because of the theoretical implications with respect to the possible origin of such chromosomal aberrations. For that reason careful attention has been given to an analy-

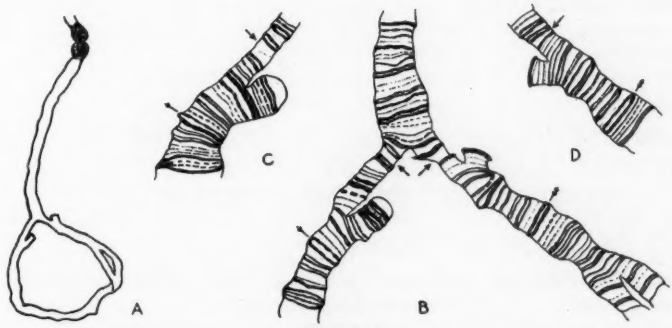


FIGURE 3

Salivary gland chromosomes showing details of terminal inversion. A—Limb of chromosome. X ca 380. B—Portion of same showing free ends of conjugated chromosomes. C and D—Similar detail from another cell. Inversion points indicated by arrows. Similar bands represented by similar symbols. X ca 1450.

sis of the banding in this inversion configuration. Details are represented in figure 3, in which the entire limb of the chromosome is shown at *A*, and a sector of the same at *B*. Additional details of the free ends of the chromosome are indicated in figures 3*C* and 3*D*, both of which are taken from another nucleus. Although complete pairing is not evident in these figures, the homologous bands may be determined readily. Their correspondence is indicated especially well in figures 3*C* and 3*D*. Free ends of the homologs seem to differ sufficiently to warrant the conclusion that they do not represent homologous regions. Moreover, in occasional figures one of the ends has been seen to be paired to the last visible band. The inversion points have been indicated by arrows.

Summary.—Four inversions have been detected in salivary gland nuclei of *D. ananassae*. Two of them occur in all hybrids between a Japanese

and an Alabama population. One of these inversions is terminal, a type previously undescribed. The two other inversions exist in a heterozygous condition in one of the parental stocks, as indicated by their occurrence in a small percentage of the glands studied.

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OBSERVATIONS ON MITOSIS IN OPALINIDS (PROTOZOA, CILIATA). I. THE BEHAVIOR AND INDIVIDUALITY OF CHROMOSOMES AND THEIR SIGNIFICANCE[1]

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1. *Introduction.*—Since it is well established that chromosomes are definite structural entities which retain their identity from one cell division to another in the Metazoa and Metaphyta, it is of interest and importance to determine whether chromosomes in Protozoa have similar individuality.

Zelleriella intermedia, a binucleate opalinid ciliate living in the rectum of a toad, was chosen for this study because of the relatively large size of the nuclei and of the chromosomes, and the gradations in size and differences in shape among the chromosomes. Furthermore, *Z. intermedia* has fewer chromosomes than certain other species of the genus.

Despite the fact that some members of the Opalinidae are particularly suited for cytological studies, little knowledge regarding their chromosomes is available; much confusion prevails and, indeed, even the very existence of the chromosomes during mitosis has been denied. The first account of mitosis in opalinids was given by Pfitzner.[2] His excellent paper, however, did not contain many details, probably because of the small size of the nuclei in *Opalina ranarum*. For nearly fifty years little new information was added, though numbers of papers have appeared from time to time. The later investigators who have reported observations on mitosis in the

opalinids include Tönniges,[3] Bezzenberger,[4] Léger and Duboscq,[5] Neresheimer,[6] Metcalf,[7] Schuster,[8] Awerinzew,[9] Sugiyama,[10] Calkins,[11] Lavier,[12] Bhatia and Gulati,[13] Fantham and Robertson,[14] Fantham,[15] Reichenow,[16] Ivanić,[17] Valkanov[18] and Nie.[19] No one appears to have made a more accurate study than Pfitzner. The conclusions reached by the majority of these investigators are now prevalent in the protozoölogical texts but are not substantiated by the present study.

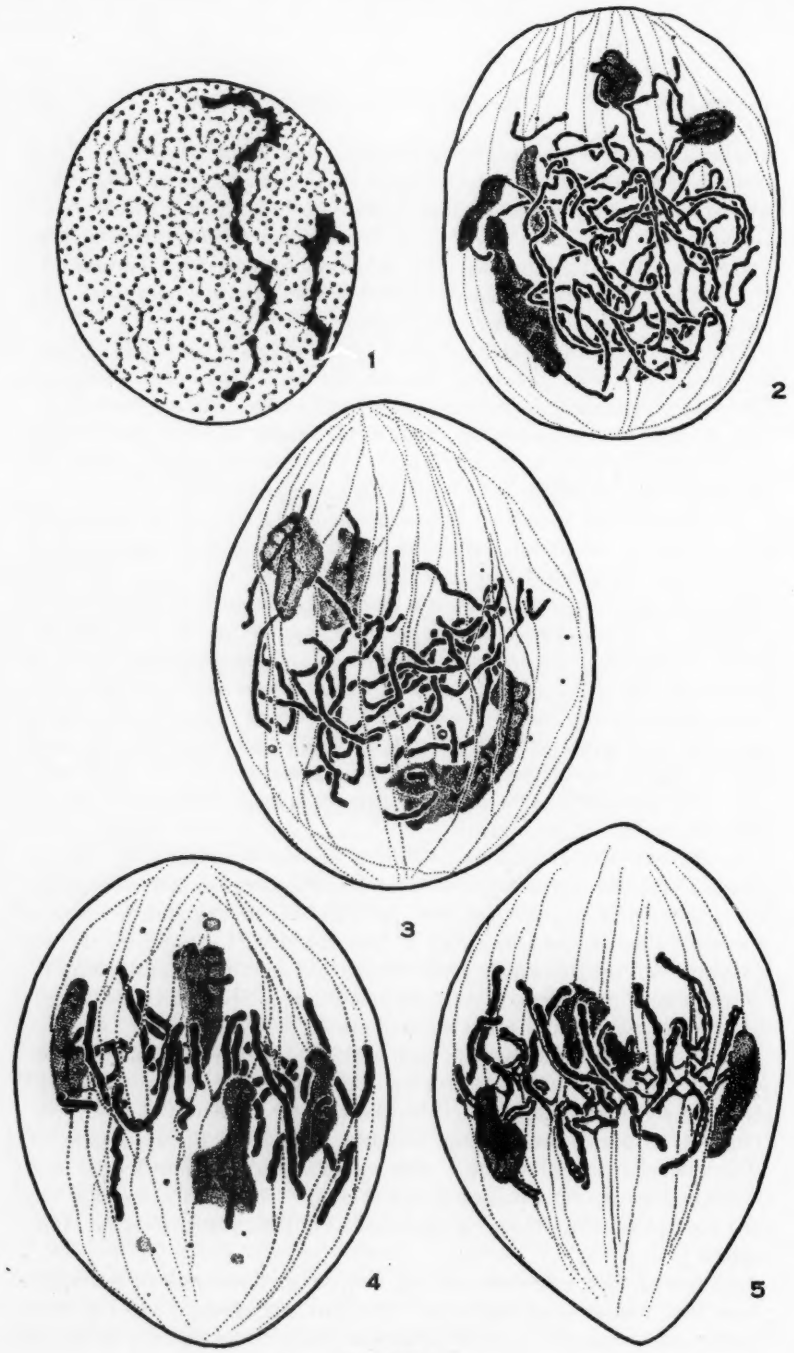
The account of mitosis in the opalinids given in this paper and the following one is more nearly complete than any previously presented. Full evidence for chromosome individuality is given for the first time for ciliates, and, to the writer's knowledge, there is no similar account for Protozoa aside from a few cases in the Sporozoa.[20] Very brief preliminary reports of the present investigation have been published elsewhere.[21]

2. *Material and Methods.*—*Zelleriella intermedia*, with which the present paper is chiefly concerned, was found in the rectum of *Bufo valliceps* from New Orleans, Louisiana. A number of other species of the genus *Zelleriella* inhabiting various anurans were also examined. Immediately after the removal of the rectum from the toads and the examination of the rectal contents, smears of these opalinids were made and fixed at once in warm Schaudinn's fluid. A number of other fixatives were also tried but since excellent results were obtained with Schaudinn's fluid containing 5% of glacial acetic acid, practically all the material studied was treated in this way. Both Feulgen's nucleal reaction and Heidenhain's iron haematoxylin were used; destaining of haematoxylin was carried on most often in a saturated aqueous solution of picric acid.

3. *Behavior of Chromosomes.*—During the resting stage (Fig. 1), the spherical or ovoid nucleus consists, as seen in the fixed and stained preparations, of three principal structures: (*a*) The nuclear membrane, which is persistent throughout mitosis, as in the majority of Protozoa; (*b*) the chromatin reticulum, which gives rise to chromosomes during mitosis; (*c*) several nucleoli which, as seen during mitosis, are constantly associated with certain portions of certain chromosomes.

Inasmuch as the two nuclei in each opalinid are alike, the following description applies to both. During early prophase (Fig. 2) the chromatin reticulum of the resting nucleus is transformed by condensation and thickening into slender individual chromosomes which have the appearance of an entangled mass of threads. The chromosomes become shorter and thicker as their condensation continues (Fig. 3). During late prophase and early metaphase (Fig. 4) they begin to collect at the equatorial region of the nucleus.

Because of their extreme condensation, the chromosomes in the metaphase are thickest and shortest. The fibre attachment area of each chromosome is usually near the equatorial plane of the nucleus, while the



FIGURES 1–9

Mitosis in *Zelleriella intermedia*. Schaud. fl., Heid. haemat. ×2 950. For explanation see text.

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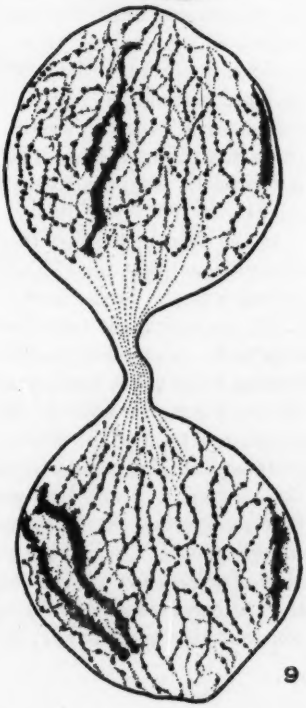

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arms of the chromosomes may extend for a considerable distance (Figs. 4 and 5). During late metaphase longitudinal splitting of the chromosomes is clearly discernible.

The separation of the chromatids in the anaphase (Fig. 5) starts at the point of the spindle fibre attachment, and hence this portion of each daughter chromosome proceeds first to the polar region of the nucleus. The shape of each individual daughter chromosome during the anaphase (Fig. 6) is definite; all the twenty-four chromosomes in this species of *Zelleriella* are atelomitic, being either *J*- or *V*-shaped, most of them being *J*-shaped.

After reaching the polar regions of the already elongated nucleus, the chromosomes remain there while the nucleus with its persistent nuclear membrane constricts at the middle (Fig. 7). The chromosomes gradually become irregular in arrangement during the telophase. The arms of various chromosomes may swing across the longitudinal axis of the nucleus instead of being nearly parallel to the axis, as is generally the case in the late anaphase. Such irregularities in arrangement become more pronounced in the late telophase (Fig. 8). The transformation of chromosomes into the chromatin reticulum involves the elongation of the telophase chromosomes, which now form anastomoses (Fig. 9). The constriction of the nucleus continues until two daughter nuclei are formed which are for a time connected by a narrow tube. As the organization of the daughter nuclei becomes more advanced, the anastomoses of the chromosomes appear to be more pronounced and the outlines of individual chromosomes become less distinct; the chromatin material becomes more evenly distributed throughout the nucleus. The connecting strand between the two daughter nuclei finally disappears, leaving two free, more or less spherical daughter nuclei.

In the light of the fact that there are well defined chromosomes in the opalinids and that their behavior is fundamentally the same as that of the chromosomes in Metazoa and Metaphyta, the statement of Calkins (1933, p. 97) regarding the organization and division of the opalinid nucleus is untenable. According to Calkins, "In *Opalina* chromatin appears to be aggregated in a few larger granules, which divide where they happen to be without further formality, the nucleus meantime assuming an indefinite division figure." The writer has studied several species of the genus *Opalina* and the results indicate that Calkins probably overlooked the chromosomes. I am also unable to confirm the report of Sugiyama[10] that in *Opalina* the chromosomes are irregular and that they divide transversely.

4. *Individuality of Chromosomes.*—Extensive studies have shown that the chromosomes (Fig. 10) can be recognized individually (1) by their constant differences in size, (2) by the location of the point of fibre attach-

ment and (3) by certain structural peculiarities such as the presence of nucleoli at definite portions of certain chromosomes. It is also evident that the chromosomes are in pairs, the members of each pair being alike. During vegetative mitosis the two members of each pair do not lie in any definite relation to each other.

The chromosome number in *Z. intermedia* is 24. This number, however, does not prevail throughout the genus. As a result of comparative studies

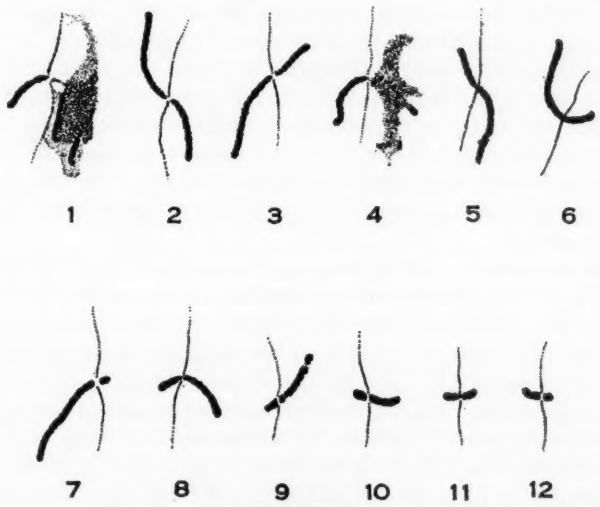

FIGURE 10

Chromosomes in *Zelleriella intermedia*. Drawn from a metaphase nucleus. A member of each chromosome pair is illustrated. Chromosome 11, because of its considerable foreshortening in this particular nucleus, is here shown semi-diagrammatically. Schaud. fl., Heid. haemat. × 2710.

of different species of *Zelleriella*, the writer has found that the number of chromosomes in certain species is much greater than 24. I am unable to confirm the statement of Metcalf (1923, p. 256) that the number of chromosomes in the zelleriellas is small (from 4 to 10). In fact, at the present time, I know of no species of *Zelleriella* having fewer than 24 chromosomes, including the species studied by Metcalf.

The 24 chromosomes in *Z. intermedia* are graded in size and there are two chromosomes for each size. There are three short pairs (already noted in the earlier reports), three long pairs and six pairs of intermediate lengths. The difference in size between some chromosome pairs is slight, between other pairs this difference is conspicuous.

The particular shape assumed by each chromosome during the anaphase is correlated with the location of the point of fibre attachment. The metaphase chromosomes (Fig. 10) have no definite shapes. All the chromosomes are atelomitic having median, sub-median or sub-terminal fibre attachments. As seen during the anaphase, nine pairs are *J*-shaped and three *V*-shaped (one of the latter is distinctly asymmetrical). A member of each pair will be described.

The three longest chromosomes (chromosomes 1, 2 and 3) consist of two *J*'s and one asymmetrical *V*, all having sub-median fibre attachments. There is very little difference in size between chromosomes 1 and 2. The short arm of chromosome 1 is approximately half as long as the long arm; in chromosome 2, however, the short arm is approximately two-thirds as long as the long arm. In chromosome 3, which is noticeably shorter than 2, the short arm is slightly less than half as long as the long arm. In the race of *Z. intermedia* from which the chromosomes (Fig. 10) were drawn a very large nucleolus is constantly found in association with the long arm of chromosome 1.

The six chromosomes of intermediate sizes consist of one asymmetrical *V* and five *J*'s. Chromosome 4 is *V*-shaped, having a sub-median fibre attachment close to the middle, the two arms being nearly equal in length. A large nucleolus is constantly present at the mid-region of the slightly longer arm of this chromosome. Chromosome 5 has a sub-median fibre attachment, the short arm being a little more than half as long as the long arm. Chromosome 6 has a sub-median fibre attachment, the short arm being only a little more than one-third as long as the long arm. Chromosome 7 is the most easily recognized of all the chromosomes, one arm being extremely short in proportion to the other, the fibre attachment being subterminal. In chromosome 8, which is much shorter than 7, the short arm is approximately half as long as the long arm, the fibre attachment being sub-median. In chromosome 9, which is shorter than 8 (very much shorter than 7), the short arm is less than one-third as long as the long arm, the fibre attachment being sub-terminal.

The three short chromosomes also differ among themselves in size and in the location of the point of fibre attachment, one being a symmetrical *V* and the other two *J*'s. Chromosome 10, which is much longer than the other two chromosomes, is *J*-shaped. Its fibre attachment is sub-median, the short arm being approximately half as long as the long arm. Chromosome 11 is a symmetrical V, having a median fibre attachment. The shortest chromosome (12) is *J*-shaped, having a sub-median fibre attachment; as in chromosome 10, the short arm is approximately half as long as the long arm.

The fact that the 12 pairs of chromosomes in *Z. intermedia* show constant and distinctive characters is significant from several points of view.

(1) It indicates constancy in the organization and individuality of chromosomes and shows qualitative and quantitative exactitude of division and distribution of chromatin material during mitosis in these protozoons, similar to that found in the Metazoa and Metaphyta.

(2) The presence of 12 pairs of chromosomes of different sizes and shapes probably means that these 24 chromosomes constitute a diploid group and that the members of each pair are homologous.

(3) It may be concluded that in the trophozoite stage, which is the longest stage in their life-cycle, the opalinids possess a diploid set of chromosomes, and that opalinids, like other ciliates, are diploid organisms in contrast with some sporozoons (such as *Aggregata*) which are haploid organisms.

[1] A large part of the work reported in this paper and the following one was carried on at the Zoölogical Laboratory, University of Pennsylvania, and it was later continued at the Osborn Zoölogical Laboratory, Yale University, where the writer held a Sterling Research Fellowship. For several summers the facilities at the Marine Biological Laboratory, Woods Hole, and the Zoölogical Laboratory of the Johns Hopkins University were used. The work was aided by a grant from the Bache Fund of the National Academy of Sciences and a grant from the University of Pennsylvania Chapter of the Society of Sigma Xi.

I am particularly indebted to Prof. D. H. Wenrich, University of Pennsylvania, for his valuable advice and criticism. I am also indebted to Prof. L. L. Woodruff, Yale University, for his interest and criticism of the manuscript and to Prof. M. M. Metcalf, who first suggested to me the studies on opalinids, for his friendly interest at all times.

More detailed reports of the present investigation will be published elsewhere.

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OBSERVATIONS ON MITOSIS IN OPALINIDS (PROTOZOA, CILIATA). II. THE ASSOCIATION OF CHROMOSOMES AND NUCLEOLI

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1. *Introduction.*—In the first paper of this series[1] the writer has described in some detail the behavior and individuality of the chromosomes of *Zelleriella intermedia*, a binucleate opalinid ciliate. It was shown that the behavior of the chromosomes in this protozoon is fundamentally the same as that found in the Metazoa and Metaphyta and that these chromosomes can be recognized individually (1) by their constant differences in size, (2) by the location of the point of fibre attachment and (3) by certain structural peculiarities such as the presence of nucleoli on certain portions of certain chromosomes. The present paper deals with the nucleoli.

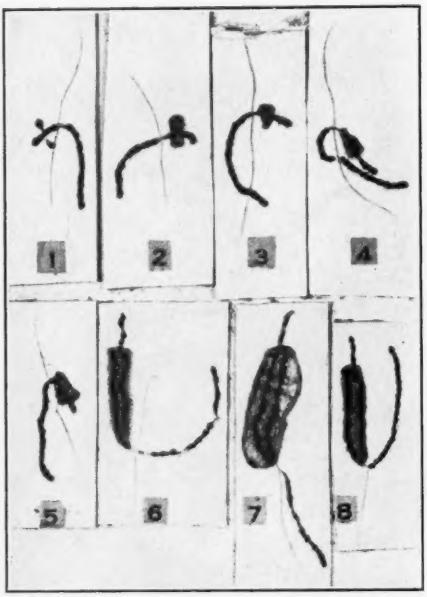
Within each of the two nuclei of *Z. intermedia*, there are, in addition to the chromosomes, conspicuous bodies—nucleoli—staining intensely with haematoxylin but not with Feulgen's reagent. The association of these nucleoli with specific portions of certain chromosomes will be described.

As far as the writer is aware, the association of chromosomes with nucleoli has never been reported in Protozoa.

2. *Manner of Association.*—The nucleoli are located on definite portions of specific chromosomes. Depending on the race, there may be 4 or 6 nucleoli formed respectively on 4 or 6 (2 or 3 pairs) of the 24 chromosomes within the nucleus. Only one nucleolus is formed on each of these chromosomes and always occupies a non-terminal position. The location is identical on the members of each chromosome pair.

If the nucleoli are heavily stained in Heidenhain's iron haematoxylin they appear as more or less homogeneous bodies, but if the stain is strongly extracted, their internal structure becomes clear. It is evident that each nucleolus actually consists of two elements—the portion of the chromosome on which (or in which) the nucleolus is located, and the nucleolar material itself (Figs. 1–8).

There is considerable variation in the appearance of this segment of the



FIGURES 1–8

Relation between chromosomes and nucleoli in *Zelleriella intermedia*. Drawn from eight different late prophase or metaphase nuclei. Schaud. fl., Heid. haemat. × 2710.

9 10 11

FIGURES 9–11

Relation between chromosomes and nucleoli in a race of *Z. intermedia*. Figures 9 and 10, chromosome pairs 1 and 4 with their nucleoli. In Figure 10 the nucleoli associated with chromosome pair 4 are fused. Figure 11, nucleoli during interkinesis. Schaud. fl., Heid. haemat. × 1355.

chromosome and in its relation to the nucleolar material. The very small nucleoli associated with chromosome pair 6 may consist merely of two granules, appearing as appendages attached to a point near the middle of the short arm (Fig. 1), reminding one of the nucleoli ("chromomere vesicles") in germ cells of certain orthoptera. The nucleoli associated with other chromosome pairs are, however, of considerable size. In these latter cases the chromosome segment appears to be either single (Fig. 10, chromosome 4) or double (Figs. 6–8). In most cases the chromosome segment shows precocious splitting, with the two chromatids clearly separated (Fig. 6). The chromatids may be far apart (Fig. 7), and even so widely separated that they approach the peripheral outline of the nucleolus (Fig. 8), with the nucleolar material located between these two chromatids. Such a condition shows a close similarity to the organization of the nucleoli in some of the Metazoa (see Dearing,[2] Figs. 27 and 28).

The definite location of the nucleoli on specific chromosomes cannot be interpreted as accidental. Their precise relationship is maintained throughout mitosis and probably retained during interkinesis although this cannot actually be observed because of the extreme attenuation of the chromosomes. In the following prophase the identical relationship is again visible.

The majority of previous investigators including Metcalf,[3] Awerinzew,[4] Tönniges,[5] Cunha and Penido,[6] Lavier,[7] Fantham and Robertson,[8] Fantham,[9] Reichenow[10] and Nie[11] have thought that the nucleoli were chromosomes and have frequently called them "chromosomes," "macrochromosomes" or "massive chromosomes." Chen,[12] Ivanić[13] and Valkanov,[14] however, have not shared this view. Obviously the nucleoli are not chromosomes nor are they a distinct set of chromosomes different from the ordinary ones. The term "macrochromosomes" is a misnomer and should be discarded entirely.

3. *The Location of Nucleoli on Specific Chromosomes.*—There are at least three races (or sub-species) of *Z. intermedia*, one race having two pairs and the others each having three pairs of chromosomes associated with nucleoli. Within each race, the location of the nucleolus on its chromosome is constant and identical for the members of each chromosome pair. The three races, all of which have been carefully studied, will be arbitrarily designated as *A*, *B* and *C*.

In race *A*, for example, the two chromosome pairs involved are 1 and 4 (Fig. 9). In chromosome 1, the very large nucleolus is located at the mid-region of the long arm, occupying approximately two-thirds of the length of this arm. In chromosome 4, the large nucleolus is located at the mid-region of the longer arm, occupying approximately three-fourths of the length of this arm.

4. *Behavior of the Nucleolus-Bearing Chromosomes and of the Nucleoli.*—

The behavior of the nucleolus-bearing chromosomes during mitosis is the same as that of the other chromosomes and the various changes take place at the same speed. Each daughter chromosome carries half of the nucleolus with it as it moves toward the pole.

During mitosis the nucleoli, probably because of their very intimate relation to the chromosomes, show a behavior similar to that of the chromosomes. For example, in race *A* the much elongated nucleoli of the resting nucleus (Fig. 11) become gradually condensed and shortened in the prophase. In the metaphase (Fig. 9), their condensation and shortening reaches a climax. In the anaphase the nucleoli are divided and the daughter nucleoli are carried to opposite poles. The division is usually equal. In the telophase the nucleoli become gradually elongated and finally greatly lengthened; this attenuated condition is retained throughout interkinesis.

5. *Appearance and Arrangement of Nucleoli in the Resting Nucleus.*—The nucleoli associated with different chromosome pairs are of different sizes, and such differences may be conspicuous. Evidently these relative sizes are retained during the resting stage. For example, in race *A*, the nucleoli associated with chromosome pair 1 are longer than those associated with chromosome pair 4 (Figs. 9 and 10). In each resting nucleus of this race two very long nucleoli and two shorter ones are regularly found (Fig. 11). Judging from the study of the history of the chromosomes and nucleoli during mitosis, it is most probable that these two long nucleoli are those associated with chromosome pair 1 and the shorter ones are those associated with chromosome pair 4. It is, therefore, possible to recognize the individual nucleoli in the resting stage and the positions of the nucleoli mark the location of the corresponding chromosomes in the resting nucleus.

The nucleoli during the resting stage are arranged at random and are located near the periphery of the nucleus. In the light of present evidence, the concept of a "mid-mitotic resting stage" as described by Metcalf (1912, 1923), Lavier (1927), Fantham (1929) and Nie (1935) is untenable. According to Metcalf (1923, p. 29), "Perhaps the most remarkable feature of the *Protoopalininae* is that in many species the nuclei are regularly found to be resting in a midmitotic condition instead of in the reticulate condition characteristic of 'resting' nuclei in general. The mitotic phase in which the nucleus comes to rest is different in different species of *Protoopalininae*. Only a few species have the nuclei characteristically in a reticulate condition. Most numerous are species whose nuclei are characteristically in an early or a late anaphase, or in an early, or a medium, or a late telophase." Again (1923, p. 252), "In no other group of animals or plants are there known nuclei which, having once entered upon mitosis, regularly come to rest without promptly completing the mitotic process."

It is evident that the nucleoli in the resting nucleus were mistaken for chromosomes by these investigators and that the chromatin reticulum was overlooked. Thus if in a resting nucleus the nucleoli happen to lie in the equatorial region (Fig. 11) such a nucleus would be considered by Metcalf and others as "resting" in the metaphase. If in a resting nucleus the nucleoli are arranged at opposite sides such a nucleus would be considered "resting" in the anaphase. The so-called "mid-mitotic resting stage" is a misinterpretation, having no factual basis. This concept must, therefore, be discarded.

6. *Real and Apparent Numbers of Nucleoli.*—It may be recalled that the real number of nucleoli associated with certain chromosomes is 4 in one race and 6 in two other races of this species of *Zelleriella*. (The real numbers of nucleoli associated with chromosomes in certain other species of this genus are 6 in one, 8 in another and 14 in still another.) However, accidental fusion of two or more nucleoli (Fig. 10, chromosome pair 4) gives rise to variations in their apparent number not only in different individuals belonging to the same race but also in the two nuclei of a single individual.

It is interesting to note that the apparent number of these nucleoli has been used by some students of Opalinidae—Metcalf (1923) and Nie (1932)—as a basis for species distinction and for establishing new species. It is now obvious that such apparent numbers are not reliable criteria for taxonomic distinctions.

7. *Fusion of Nucleoli.*—If two or more nucleoli are close to each other, they may clump together or even fuse into a single body. Fusion does not take place in a definite pattern. There is no special attraction between the nucleoli of homologous chromosomes. The fusion of nucleoli appears to be accidental and it may occur in only one of the two nuclei of an individual opalinid. The fusion may be only temporary in which case the individual nucleoli separate in the anaphase.

8. *Discussion.*—The nucleoli of these opalinids are similar to those of some Metazoa (Dearing[2]) in the following three respects: (1) The nucleoli are constantly associated with specific portions of specific chromosomes. (2) The nucleolar material reacts negatively to Feulgen's test whereas the portion of the chromosome on which it is located is definitely colored by the reagent. (3) According to Dearing, in *Ambystoma* each nucleolus is formed between the two widely separated chromatids. In these opalinids, as already described, the chromosome segment on which the nucleolus is located may appear single or double; the latter is more often the case. The two chromatids may be so widely separated that they approach the peripheral outline of the nucleolus, as in the case of *Ambystoma*.

The difference between the nucleoli in these opalinids and those of Metazoa and Metaphyta lies in the fact that the nucleolar material does not disappear during mitosis in these ciliates. In distantly related groups of

animals and plants one can hardly expect the nuclear structure and modes of nuclear division to be identical in every respect. Even among the Protozoa great diversities in nuclear structure and division are manifest. Centrosomes, e.g., are present in some forms but not in others. The nuclear membrane is persistent throughout mitosis in the majority of forms but not in all of them. Variations also exist in the appearance and location of chromatin during interkinesis, and in the location and behavior of nucleolar material (Bělař[15]). The regular disappearance of nucleoli during the prophase in some protozoons such as *Euglypha* (Bělař[15]) and the persistence of nucleoli in these opalinids is another manifestation of the great range of variations to be expected. If the figures of Bezzenberger[16] are accurate, it is possible that the nucleoli in some opalinids regularly disappear during mitosis.

Is the nucleolar material in these opalinids identical with the macrochromatin present in the macronuclei of other ciliates? That it is has been the conclusion of several previous investigators, including Metcalf (1914, 1923), Tönniges[5] and Nie (1935) who have called the nucleoli "macrochromatin" and "macrochromosomes." It is impossible to consider the nucleolar material in the opalinids as identical with the macrochromatin of other ciliates for the following reasons:

(1) In the ciliates the macrochromatin is differentiated from the chromatin of one or more early descendants of a zygote nucleus whereas nothing is known of the origin of the nucleolar material in the opalinids. (2) Following conjugation the macrochromatin of ciliates disintegrates and is extruded into the cytoplasm. "Extrusion of chromatin" in the opalinids has been reported by previous investigators but their description is far from convincing. No such phenomenon has been proved to occur in the opalinids. (3) Feulgen's reagent does not color the nucleolar material of the opalinids, whereas the macrochromatin of ciliates from widely separated groups consistently gives a positive reaction.

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PROGRESSIVE WAVES OF FINITE AMPLITUDE AND SOME STEADY MOTIONS OF AN ELASTIC FLUID

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1. *Progressive Plane Waves.*—When a gas is initially stationary and at uniform temperature and pressure the density ρ may be regarded as also uniform initially with a value ρ_0 which for convenience may be taken as unity. The velocity of sound at this time will also be independent of position and equal, say, to c_0.

If now the gas is set in motion by a piston so that it moves parallel to the x-axis, then at any subsequent time t the pressure p at the place x will depend only on the density so long as no shock waves have passed over this place and the velocity potential ϕ will satisfy the partial differential equation

$$\frac{\partial^2\phi}{\partial t^2} + 2\frac{\partial\phi}{\partial x}\frac{\partial^2\phi}{\partial x\partial t} + \left(\frac{\partial\phi}{\partial x}\right)^2\frac{\partial^2\phi}{\partial x^2} = c^2\frac{\partial^2\phi}{\partial x^2} \qquad (1)$$

which is a particular case of a more general equation given by Lagrange.[1]

It is known that this equation may be treated with the aid of the well-known contact transformation

$$X = \frac{\partial\phi}{\partial x} \equiv u,\ T = \frac{\partial\phi}{\partial t} = f'(\rho) - \frac{1}{2}u^2 \qquad (2)$$

$$W = xX + tT - \phi = ux + t[f'(\rho) - \frac{1}{2}u^2] - \phi \qquad (3)$$

usually associated with the name of Legendre[2] but apparently known also to Euler[3] and Monge.[4] It is thus converted into a linear partial differential equation

$$\frac{\partial^2 W}{\partial X^2} - 2X\frac{\partial^2 W}{\partial X\partial T} + X^2\frac{\partial^2 W}{\partial T^2} = c^2\frac{\partial^2 W}{\partial T^2} \qquad (4)$$

which, as Poisson remarks,[5] is of a type which, when c is constant, can be solved by means of definite integrals but the resulting expression does not

readily give the type of solution corresponding to a progressive wave which he found by an ingenious direct method of solution involving the use of an inequality to show that one part of the complete solution is zero in a progressive wave.

This apparent failure of Legendre's transformation to give all the solutions of the partial differential equation arises from the fact that X and T are not independent quantities in the progressive wave and so cannot be treated as new independent variables for the formation of a new partial differential equation. The solution for the progressive wave is not a singular solution in the sense in which this term is usually used;[6] the nature of the solution is discussed by McCowan[7] who regards it as special, i.e., a limiting case of the general solution.

The failure is only apparent because the relation

$$dW = xdX + tdT \tag{5}$$

which is true in any case, shows that when X and T are functions of a single parameter τ, W is also a function of τ and we have the equations

$$\phi = xX(\tau) + tT(\tau) - W(\tau) \tag{6}$$

$$0 = xX'(\tau) + tT'(\tau) - W'(\tau) \tag{7}$$

which are precisely those which define the progressive waves. The equations may be written in other forms and we shall find it convenient to indicate other ways of finding them.

If x_0 is the initial coördinate of the particle which is at x at time t we may write

$$\rho = \frac{\partial x_0}{\partial x}, \quad \rho u = -\frac{\partial x_0}{\partial t} \tag{8}$$

and the partial differential equation for x_0 is

$$\left(\frac{\partial x_0}{\partial x}\right)^2 \frac{\partial^2 x_0}{\partial t^2} - 2\frac{\partial x_0}{\partial x}\frac{\partial x_0}{\partial t}\frac{\partial^2 x_0}{\partial x \partial t} + \left(\frac{\partial x_0}{\partial t}\right)^2 \frac{\partial^2 x_0}{\partial x^2} = c^2 \frac{\partial^2 x_0}{\partial x^2}\left(\frac{\partial x_0}{\partial x}\right)^2. \tag{9}$$

This may also be reduced to a linear equation by the transformation of Legendre, the appropriate equations being

$$\rho = \frac{\partial x_0}{\partial x}, \; m = \rho u = -\frac{\partial x_0}{\partial t}, \; \sigma = \rho(x - ut) - x_0 \tag{10}$$

$$\rho^2 \frac{\partial^2 \sigma}{\partial \rho^2} - 2m\rho \frac{\partial^2 \sigma}{\partial \rho \partial m} + m^2 \frac{\partial^2 \sigma}{\partial m^2} = \rho^2 c^2 \frac{\partial^2 \sigma}{\partial m^2}. \tag{11}$$

The solution corresponding to progressive waves is now

$$x_0 = \rho(\tau)[x - tu(\tau)] - \sigma/\tau) \tag{12}$$

where τ is defined by the equation

$$0 = \rho'(\tau)[x - tu(\tau)] - t\rho(\tau)u'(\tau) - \sigma'(\tau). \tag{13}$$

This equation must be the same as before because a relation between ρ and ρu implies a relation between the quantities X and T given by equations (2). Comparing (13) with (7), which may be written in the form

$$0 = xu'(\tau) - t[u(\tau)u'(\tau) + c^2(\tau)\rho'(\tau)/\rho(\tau)] - W'(\tau) \tag{14}$$

we find that $$c^2(\tau)\,\rho'^2(\tau) = \rho^2(\tau)\,u'^2(\tau).$$

This is precisely the condition that the quantity x_0 defined by (12) should be a solution of the partial differential equation (9). A comparison of (13) and (14) also gives the relation

$$\rho'(\tau)W'(\tau) = u'(\tau)\sigma'(\tau). \tag{15}$$

It should be noticed that when x is taken as dependent variable (9) transforms into Lagrange's differential equation[8]

$$\left(\frac{\partial x}{\partial x_0}\right)^2 \frac{\partial^2 x}{\partial t^2} = c^2 \frac{\partial^2 x}{\partial x_0^2} \tag{16}$$

which has been frequently treated with the aid of Legendre's transformation.[9] The solution for progressive waves is now

$$\begin{aligned} x &= tu(\tau) + xv(\tau) - w(\tau) \\ 0 &= tu'(\tau) + xv'(\tau) - w'(\tau) \end{aligned} \tag{17}$$

where $v(\tau)$ is the reciprocal of $\rho(\tau)$. We have in fact

$$u = \frac{\partial x}{\partial \tau}, \quad v = \frac{\partial x}{\partial x_0} = \frac{1}{\rho}. \tag{18}$$

A comparison of (17) and (12) shows at once that $\rho(\tau)w(\tau) = -\sigma(\tau)$. Indeed, in the Legendre transformation in which u and v are new independent variables the new dependent variable is $w = -v\sigma = -\sigma/\rho$.

If we introduce the variable ψ which Kirchhoff calls the stream-function[10] and write

$$x_0 = \frac{\partial \psi}{\partial x}, \quad \rho = \frac{\partial^2 \psi}{\partial x^2}, \quad \rho u = -\frac{\partial^2 \psi}{\partial x \partial t}, \quad p + \rho u^2 = \frac{\partial^2 \psi}{\partial t^2}, \tag{19}$$

the equation for ψ involves only the second derivatives and may be treated by the method of Legendre[11] in which the equation is first solved for the derivative $\dfrac{\partial^2 \psi}{\partial t^2}$ and then differentiated with respect to x. The result is, of course, equation (9). If, on the other hand, the equation for ψ is first sub-

jected to a Legendre transformation in which the quantities $x_0 = \frac{\partial \psi}{\partial x}$ and $y_0 = \frac{\partial \psi}{\partial t}$ are taken as new independent variables, the new equation is of the same type as before, involving only second derivatives of the new dependent variable z. When this equation is treated by Legendre's method of differentiation so as to provide an equation that can be transformed into a linear equation by Legendre's transformation the new dependent variable is $\frac{\partial z_0}{\partial y_0}$ which is t and since

$$\frac{\partial t}{\partial x_0} = \frac{u}{p}, \quad \frac{\partial t}{\partial y_0} = \frac{1}{p} \tag{20}$$

a relation between $\frac{\partial t}{\partial x_0}$ and $\frac{\partial t}{\partial y_0}$ implies a relation between p and u. The solution for progressive waves is thus obtained in the form

$$t = [y_0 + x_0 u(\tau)]/p(\tau) - s(\tau) \tag{21}$$

where τ is defined by the equation

$$u'(\tau)x_0 = tp'(\tau) + p'(\tau)s(\tau) + p(\tau)s'(\tau) \tag{22}$$

which is equivalent to one derived from (12) and (13) by eliminating x.

If, in the differential equation for t we take y_0 as new dependent variable, the new independent variables being x_0 and t, we obtain the equation

$$\frac{\partial^2 y_0}{\partial t^2} = c^2\rho^2 \frac{\partial^2 y_0}{\partial x_0^2}. \tag{23}$$

This equation may be treated by Legendre's transformation, the new independent variables being

$$\frac{\partial y_0}{\partial t} = p, \quad \frac{\partial y_0}{\partial x_0} = -u \tag{24}$$

while the new dependent variable is $tp - ux_0 - y_0 = -ps$. Calling this quantity R we have the equations

$$\frac{\partial^2 R}{\partial u^2} = c^2\rho^2 \frac{\partial^2 R}{\partial p^2} \tag{25}$$

$$dR = tdp - x_0 du$$

and in adiabatic flow the partial differential equation can be reduced to the equation of Euler and Poisson[12] and treated by the method of Riemann.

The wave-solution given by the failure of Legendre's transformation is obtained again in the form (21).

The wave-solutions have been discussed at some length by Hugoniot[13] and Hadamard,[14] particular attention being paid to the generation of the wave-motion from a state of rest by the accelerated motion of a piston and the formation of singularities or discontinuities in the motion as indicated by Stokes.[15] By means of a representation in a space of three dimensions the solution is interpreted as a developable surface which touches the plane $z = 0$ along a characteristic of the partial differential equation. The edge of regression of this developable indicates where singularities occur, for beyond this edge z becomes non-existent or two-valued. If $x = \zeta(\sigma)$, $y = \eta(\sigma)$, $z = \rho(\sigma)$ are the equations of the edge the developable can be regarded as the envelope of the plane

$$[x - \zeta(\sigma)][\eta'(\sigma)\rho''(\sigma) - \rho'(\sigma)\eta''(\sigma)] + [y - \eta(\sigma)][\rho'(\sigma)\zeta''(\sigma) - \zeta'(\sigma)\rho''(\sigma)] + [(z - \rho(\sigma)][\zeta'(\sigma)\eta''(\sigma) - \eta'(\sigma)\zeta''(\sigma)] \tag{26}$$

and the equation obtained by differentiating with respect to σ keeping x, y, z constant can be expressed in various forms which are all included in the equations

$$\frac{x - \zeta(\sigma)}{\zeta'(\sigma)} = \frac{y - \eta(\sigma)}{\eta'(\sigma)} = \frac{z - \rho(\sigma)}{\rho'(\sigma)} \tag{27}$$

obtained by combining any one form of the equation with (26).

This result will be used in §2; it is mentioned here because the general results of Hugoniot and Hadamard for the case of the equation of wave-propagation are applicable also to the equation for steady two-dimensional flow, the solution for progressive waves being replaced by a solution giving a generalization of the well-known flow discussed by Prandtl and Meyer. A few words must be said about a type of solution of the equation for ψ in which the second derivatives of ψ are all related and are thus functions of a single variable s. The general solution for such a case which will be derived in §2, gives the relations

$$\begin{aligned} x_0 &= \frac{\partial \psi}{\partial x} = e(s)x + st + n(s), \quad 0 = e'(s)x + t + n'(s) \\ y_0 &= \frac{\partial \psi}{\partial t} = sx + t\int ds/e'(s) + \int n'(s)ds/e'(s) \end{aligned} \tag{28}$$

and in the adiabatic flow in which $p = k\rho^\gamma$ we have

$$\begin{aligned} s &= Ce(s) - \frac{2}{\gamma - 1}\sqrt{k\gamma}\left[e(s)\right]^{\frac{\gamma+1}{2}} \\ \rho = e(s), \quad \rho u &= -s, \quad p + \rho u^2 = \int ds/e'(s), \end{aligned} \tag{29}$$

where C is an arbitrary constant.

It may be remarked that Lagrange's equation (16) is derivable by differentiation with respect to x_0 from an equation of type

$$\frac{\partial^2 w}{\partial t^2} = F\left(\frac{\partial^2 w}{\partial x_0^2}\right) \tag{16'}$$

wherein $x = \dfrac{\partial w}{\partial x_0}$, $F'(z) = c^2(z)/z^2$. This equation had a particular solution of type

$$\frac{\partial w}{\partial t} = t\int ds_0/e_0'(s_0) + s_0 x_0 + \int n_0'(s_0)ds_0/e_0'(s_0),\ 0 = t + x_0 e_0'(s_0) + n_0'(s_0),\ \frac{\partial w}{\partial x_0} = s_0 t + x_0 e_0(s_0) + n_0(s_0) \text{ if } \int ds_0/e_0'(s_0) = F[e_0(s_0)].$$

In adiabatic flow

$$\sqrt{k\gamma}\left(\frac{1-\gamma}{2}\right)^{-1}\left[e_0(s_0)\right]^{\frac{1-\gamma}{2}} = C \pm s_0$$

$$\frac{1}{\rho} = e_0(s_0),\quad c = \sqrt{k\gamma}\left[e_0(s_0)\right]^{\frac{1-\gamma}{2}}.$$

2. *Steady Flow in Two Dimensions.*—The dynamical equations

$$\begin{aligned}\frac{\partial}{\partial x}(\rho u^2 + p) + \frac{\partial}{\partial y}(\rho uv) &= 0\\ \frac{\partial}{\partial x}(\rho uv) + \frac{\partial}{\partial y}(\rho v^2 + p) &= 0\end{aligned} \tag{30}$$

can be satisfied in a general way by writing

$$\rho u^2 + p = -\frac{\partial^2 S}{\partial y^2},\ \rho uv = \frac{\partial^2 S}{\partial x \partial y},\ \rho v^2 + p = -\frac{\partial^2 S}{\partial x^2} \tag{31}$$

where S is a function which Neumann[16] calls the stream-function but which will be called here the stress-function. It must be chosen so that the equation of continuity

$$\frac{\partial}{\partial x}(\rho u) + \frac{\partial}{\partial y}(\rho v) = 0 \tag{32}$$

is satisfied and that p is a specified function of ρ.

In the Prandtl-Meyer type of flow, the existence of which follows from the general remarks made by Hadamard[14] on the solution of equations of type

$$A\left(\frac{\partial\phi}{\partial x}, \frac{\partial\phi}{\partial y}\right)\frac{\partial^2\phi}{\partial x^2} + 2H\left(\frac{\partial\phi}{\partial x}, \frac{\partial\phi}{\partial y}\right)\frac{\partial^2\phi}{\partial x\partial y} + B\left(\frac{\partial\phi}{\partial x}, \frac{\partial\phi}{\partial y}\right)\frac{\partial^2\phi}{\partial y^2} \tag{33}$$

the quantities p, ρ, u and v are all functions of a single parameter τ and so there must be solutions of Neumann's equation for S in which the second derivatives of S are all functions of a single parameter s. Replacing S, for the moment by z and using r, s, t for the second derivatives $\frac{\partial^2 z}{\partial x^2}, \frac{\partial z}{\partial x\partial y}$, $\frac{\partial^2 z}{\partial y^2}$, respectively, we may ask under what condition the equations $r = e(s)$, $t = f(s)$ are compatible. Denoting third derivatives by k, l, m, n we find by differentiating

$$k = l'(s)l, \quad l = e(s)m, \quad m = f'(s)l, \quad n = f'(s)m \tag{34}$$

and the condition of compatibility is

$$e'(s)f'(s) = 1. \tag{35}$$

It will be noticed that we have identified the parameter s with $\frac{\partial^2 z}{\partial x\partial y}$. But this can be done without much loss of generality.

The equations $r = e(s)$, $t = f(s)$ may be regarded as partial differential equations of the first order for $\frac{\partial S}{\partial x}$ and $\frac{\partial S}{\partial y}$, respectively. Solving these we have

$$\begin{aligned}\frac{\partial S}{\partial x} &= xe(s) + ys + g(s)\\ \frac{\partial S}{\partial y} &= xs + yf(s) + h(s)\end{aligned} \tag{36}$$

where s is given respectively by the equations

$$\begin{aligned}0 &= xe'(s) + y + g'(s)\\ 0 &= x + yf'(s) + h'(s).\end{aligned} \tag{37}$$

These must be the same and so $h'(s) = f'(s)g'(s)$, $f'(s)e'(s) = 1$.

[Though the analysis of Hadamard gives some of the general features of the flow specified by these equations and the flow has already been studied in a general way by S. Lees[17] and the present author,[18] the analysis can now be presented in an improved form which indicates more clearly its relation to the graphical method of solving problems in supersonic flow used by Prandtl,[19] Ackeret[20] and Busemann.[21] The improvements depend upon advantageous choices of the parameter τ on which the principal quantities

depend. In the above form of the solution $\tau = s$ where s represents the physical quantity ρuv. We shall now choose τ so that the velocity of sound is the function $c(\tau)$ which will be regarded as the derivative of a function $a(\tau)$ so that $c(\tau) = a'(\tau)$.]

The parameter τ is, however, not yet defined because the functions are unspecified. We shall suppose further that the components of velocity u, v, are functions $u(\tau), v(\tau)$ given by the equations

$$u(\tau) = a(\tau)\cos\tau - a'(\tau)\sin\tau, \quad v(\tau) = a(\tau)\sin\tau + a'(\tau)\cos\tau \tag{38}$$

and supposed not to be constants
then

$$u'(\tau) = -\sin\tau[a(\tau) + a''(\tau)], \; v'(\tau) = \cos\tau[a(\tau) + a''(\tau)]. \tag{39}$$

The velocity potential ϕ may now be expressed in the form

$$\phi = xu(\tau) + yv(\tau) - w(\tau) \tag{40}$$

where τ is defined by the equation

$$0 = xu'(\tau) + yv'(\tau) - w'(\tau). \tag{41}$$

Lagrange's partial differential equation for ϕ, when written in Rayleigh's form

$$c^2\left(\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y}\right) = u\left(u\frac{\partial u}{\partial x} + v\frac{\partial v}{\partial x}\right) + v\left(u\frac{\partial u}{\partial y} + v\frac{\partial v}{\partial y}\right) \tag{42}$$

is seen to be satisfied on account of the equation $c = a'(\tau)$ provided $u''(\tau)$, $v''(\tau)$ and $w''(\tau)$ are not all zero, for then we may write

$$M\frac{\partial\tau}{\partial x} \equiv u'(\tau), \; x\frac{\partial\tau}{\partial y} = v'(\tau), \quad M = w''(\tau) - xu''(\tau) - yv''(\tau) \tag{43}$$

where M is different from zero.

Since $q^2 = u^2 + v^2 = a'^2(\tau) + a^2(\tau)$, we have $a^2(\tau) = q^2 - c^2$ and so $q^2 \geq c^2$, a result obtained before. Writing $\rho = \rho(\tau)$, the equation of continuity (31) gives

$$\rho'(\tau)a'(\tau)[a(\tau) + a''(\tau)] + \rho(\tau)[a(\tau) + a''(\tau)]^2 = 0 \tag{44}$$

and since, by hypothesis u and v are not both constant, we have $a(\tau) + a''(\tau) \neq 0$ and so

$$\rho'(\tau)a'(\tau) + \rho(\tau)a''(\tau) + \rho(\tau)a(\tau) = 0. \tag{45}$$

In adiabatic flow the relation between c and q is

$$\frac{2c^2}{\gamma - 1} + q^2 = b^2 \tag{46}$$

where b is a constant. This gives the equation

$$a^2(\tau) + \frac{\gamma+1}{\gamma-1} a'^2(\tau) = b^2 \tag{47}$$

whose solution is

$$a(\tau) = b \sin(\lambda\tau + \alpha) \tag{48}$$

where α is a constant and $\lambda = \left(\frac{\gamma-1}{\gamma+1}\right)^{1/2}$.

To obtain the stream-lines we put

$$x = R\cos\tau - T(\tau)\sin\tau, \quad y = R\sin\tau + T(\tau)\cos\tau. \tag{49}$$

Substituting in (41) we find that

$$\begin{aligned} w'(\tau) &= T(\tau)[a(\tau) + a''(\tau)], \quad \phi = Ra(\tau) + T(\tau)a'(\tau) - w(\tau) \\ dx &= [dR - T(\tau)d\tau]\cos\tau - [R + T'(\tau)]\sin\tau d\tau \\ dy &= [dR - T(\tau)d\tau]\sin\tau + [R + T'(\tau)]\cos\tau d\tau. \end{aligned} \tag{50}$$

The equation for the stream-lines

$$\frac{dy}{dx} = \frac{v}{u} = \frac{a(\tau)\sin\tau + a'(\tau)\cos\tau}{a(\tau)\cos\tau - a'(\tau)\sin\tau} \tag{51}$$

thus takes the form

$$a'(\tau)[dR - T(\tau)d\tau] = a(\tau)[R + T'(\tau)]d\tau. \tag{52}$$

Making use of (45) it becomes

$$d[R\rho a'(\tau)] = \rho d[T(\tau)a(\tau)]. \tag{53}$$

Putting $T(\tau)a(\tau) = h'(\rho)$ we have

$$R\rho c = \rho h'(\rho) - h(\rho) + n \tag{54}$$

where n is a constant associated with the particular stream-line under consideration. The equations of the stream-line may thus be expressed in the parametric form

$$\begin{aligned} x &= \frac{\cos\tau}{a'(\tau)}\left[h'(\rho) - \frac{1}{\rho}h(\rho) + \frac{n}{\rho}\right] - \frac{\sin\tau}{a(\tau)}h'(\rho) \\ y &= \frac{\sin\tau}{a'(\tau)}\left[h'(\rho) - \frac{1}{\rho}h(\rho) + \frac{n}{\rho}\right] + \frac{\cos\tau}{a(\tau)}h'(\rho) \end{aligned} \tag{55}$$

where ρ is a function of τ given by equation (45). We may also write

$$\psi = \rho c[x\cos\tau - y\sin\tau] + h(\rho) - \rho h'(\rho). \tag{56}$$

It should be noticed that the points on different stream-lines that have the same value of τ all lie on a straight line

$$0 = xu'(\tau) + yv'(\tau) - w'(\tau) \tag{57}$$

which cuts each stream-line at the Mach angle β corresponding to this value of τ. Equations (55) indicate that

$$\tan \beta = \frac{a'(\tau)}{a(\tau)}, \qquad \sin \beta = \frac{c}{q} \tag{58}$$

and that the distance d along this line between two stream-lines with parameters n_1 and n_2 is

$$d = \frac{n_1 - n_2}{\rho a'(\tau)} = \frac{n_1 - n_2}{\rho c}. \tag{59}$$

Thus $d\rho c$ is constant for the stream-lines. This relation, when written in the form $d\rho q \sin \beta = \text{constant}$, is merely another way of expressing the equation of continuity.

The lines $\tau = \text{constant}$ generally have an envelope E as indicated in Ackeret's diagram, reproduced with full explanations by Taylor and McColl.[22] This envelope may be regarded as the projection of the edge of the developable obtained by regarding ϕ as a third coördinate z and marks out a boundary of the region in which the flow can be represented by this solution of the equations. Taking σ to be the length of the arc of E measured from some fixed point, we have $\zeta'(\sigma) = \cos \tau$, $\eta'(\sigma) = -\sin \tau$, $r\zeta''(\sigma) = -\sin \tau$, $r\eta''(\sigma) = \cos \tau$, where r is the radius of curvature at the point σ on E. Comparing (40) and (41) with (26) and (27) we obtain the relations $a(\tau) = -\rho'(\sigma)$,

$$\begin{aligned} w'(\tau) &= [a(\tau) + a''(\tau)][\eta(\sigma) \cos \tau - \zeta(\sigma) \sin \tau] \\ &\quad - X[a(\tau) + a''(\tau)] \end{aligned} \tag{60}$$

$$w(\tau) = Y\rho'(\sigma) + rX\rho''(\sigma) - \rho(\sigma)$$

where X and Y are the distances of the origin of coördinates from the tangent and normal at the point σ on E. If, on the other hand, we compare (40) and (41) with (36) and (37) we obtain the relations

$$e'(s) = -\tan \tau, \quad f'(s) = -\cot \tau, \; ds/d\tau = \rho'(\tau)q^2(\tau) \sin \tau \cos \tau. \tag{61}$$

3. *Spiral Flow.*—Keeping the notation of §2 and using polar coördinates (q, ω) in the uv-plane as independent variables, Tschapliguine[23] obtained linear partial differential equations for ϕ and ψ the solutions of which when expanded in series of particular solutions involve linearly a set of constants. We now wish to know how these constants of integration enter into the expression for the stress-function S. It has been found

that in the case of the fluid motion which Taylor[24] calls spiral flow S is a homogeneous quadratic function of the constants. We have in fact

$$\phi = m\omega - n\int q\,dq\,Q'/Q^2, \quad \psi = n\omega - m\int Q\,dq/q^2$$

$$S = -mn\omega + \int dq\left[\frac{m^2p}{q^3} + \frac{n^2pQ'}{Q^3} + \frac{n^2qQ'}{Q^2}\right] \tag{62}$$

$$\frac{\partial S}{\partial x} = mn\frac{y}{r^2} - pk - \frac{n^2x}{pr^2}, \quad \frac{\partial S}{\partial y} = -mn\frac{x}{r^2} - py - \frac{n^2y}{pr^2}$$

$$\varphi \equiv \arctan(y/x) = \omega - \arctan(mQ/nq),$$

$$r^2 \equiv x^2 + y^2 = \frac{m^2}{q^2} + \frac{n^2}{Q^2}$$

where m and n are the constants and $Q = \rho q$.

A diagram in the Qq-plane may be used with advantage in the study of this and other types of flow. The curve C giving the relation between

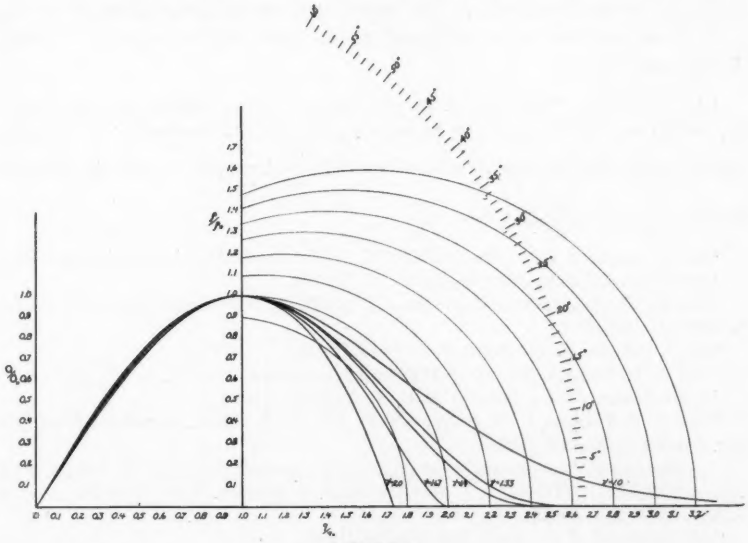

FIGURE 1

Q and q in adiabatic flow starts from O, runs up to a maximum value of Q and then slopes downward until a point is reached at which Q and ρ are both zero. If now a tangent at a point P on this curve meets the axis of q in T, the circle on OT as diameter meets the ordinate PN in a point R such that the angle between OR and Oq is Mach's angle β. We have in fact

$$q^2/c^2 = 1 - qQ'/Q, \; p = -\int Qdq, \quad \rho = Q/q, \qquad (63)$$

where Q' is the derivative of the function $Q(q)$. The velocity of sound c is represented as the distance from OR of N, the foot of the ordinate of P. The pressure p is represented by the area between this ordinate and the portions of the curve and q-axis which meet at the point where the density is zero.

Figure 1, which shows the form of the Q—q curves for adiabatic flow for different values of γ has been drawn for me by Francis and Milton Clauser. Some of the circles have been drawn and a scale included so that the Mach angle can be read off by placing a straight edge through the origin and the point R on the circle. (The points are not marked on the diagram.) In spiral flow the curves along which ω is constant may be obtained by a rotation of the curve C for we have the equations

$$x \cos \omega + y \sin \omega = n/Q, \quad x \sin \omega - y \cos \omega = m/q. \qquad (64)$$

The transverse velocity and the density of radial momentum are both inversely proportional to r. The moment of momentum about O of any portion of the fluid is proportional to its mass and so remains constant during motion.

[1] J. L. Lagrange, "Oeuvres," t. 12, pp. 323-340. If the relation between p and ρ is $p = f(\rho) - \rho f'(\rho)$ we have $c^2 = dp/d\rho = -\rho f''(\rho)$ and the equation for ϕ is obtained by eliminating ρ between the equation of continuity $\frac{\partial \rho}{\partial t} + \frac{\partial}{\partial x}(\rho u) = 0$ and the equation of motion $\frac{\partial}{\partial t}(\rho u) + \frac{\partial}{\partial x}(\rho u^2) = -c^2 \frac{\partial \rho}{\partial x}$.

[2] A. M. Legendre, *Mém. Paris* (1787) 347, L'Histoire de l'Acad. des sciences (1787).

[3] See P. Stäckel, *Bibl. Math.* (3) **1**, 275, 517 (1900).

[4] See M. Chasles, Aperçu Historique . . . , p. 376, A. de Morgan, *Camb. Phil Trans.*, **8**, 606–613 (1848).

[5] S. D. Poisson, *J. Éc. Polyt.*, **7**, 312–392 (1808).

[6] See A. R. Forsyth, Theory of Differential Equations, Part IV, vol. 6, p. 3.

[7] J. McCowan, *Proc. Edinburgh Math. Soc.*, **11**, 2–6 (1893).

[8] See. S. D. Poisson, *J. Éc. Polyt.*, cah. 21, 187–204 (1832), S. Earnshaw, *Proc. Roy. Soc. London*, **9**, 500–501 (1858).

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[11] A. M. Legendre, loc. cit.; J. R. Wilton, *Mess. of Math.*, **43**, 58–63 (1914). Wilton solves an equation from which (16) may be derived by differentiation, the dependent variable θ being such that $x = \frac{\partial \theta}{\partial x_0}$.

[12] See Darboux, "Théorie des surfaces," t. 2, chapters 3, 4 and 9. Another method of treating the linear differential equations derived from (16) and (23) by Legendre's transformation is given by R. Liouville, *Comptes Rendus*, **98**, 723–726 (1884).

[13] H. Hugoniot, *J. Éc. Polyt.*, cah. 57, 1–97 (1887), cah. 58, 1–125 (1889).

[14] J. Hadamard, loc. cit., "Cours d'Analyse," t. 2.

[15] G. G. Stokes, *Phil. Mag.*, (3) **23**, 349 (1848); "Math. Phys. Papers," vol. 2, 51–55. A good illustration of the formation of a shock wave is given by W. Hope-Jones, *Math. Gazette*, **14**, 173–186(1928–9).

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[24] G. I. Taylor, *J. London Math. Soc.*, **5**, 224–240 (1930).